Nucleic Acid Isolation and Purification

4th edition
Sea Otters

Sea otters are the smallest marine mammals and are adapted to spending almost all of their lives in the ocean. Although they resemble small seals, sea otters are not actually related to seals, but rather to weasels, badgers and minks. They belong to the species “Enhydra lutris”, from the Greek “en hydra”, meaning “in the water”, and the Latin “lutris”, meaning otter.

Sea otters can be found in the Pacific Ocean along the coast of California, up to Alaska, along the east coast of Russia, and all the way up to the northern tip of Japan. They prefer coastal waters and only swim out into the deep ocean when travelling between islands and the mainland.

Unlike other marine mammals such as seals and dolphins, sea otters do not rely on a layer of fat to insulate their bodies from the cold water. Instead, sea otters depend on their fur to keep warm. But even with a thick fur coat, the sea otter has to work hard to keep warm, spending hours each day grooming its fur. Any water needs to be squeezed out and air needs to be blown into the fur. The air makes the fur waterproof, ensuring that the otter’s skin remains warm and dry. That’s why cleaning is essential. Dirty fur can easily get wet and place the otter at risk of dying from the cold.

In addition to their thick coats, sea otters have another defense against the cold ocean water. The otter has a high body temperature (around +38°C), which requires a fast metabolic rate. As a result, sea otters need to eat a lot, sometimes as much as 25% of their body weight each day. This is the equivalent of humans eating about 100 hamburgers!

Sea otters eat a variety of foods, including shellfish, sea urchins, fish and many different types of invertebrate sea life. As they must constantly eat to survive, otters spend a lot of time diving for food. Otters can dive up to 100 m for several minutes, although they prefer shallow waters where they can make quicker dives instead.

Capturing their prey is not always an easy task. However, the sea otter is a highly intelligent animal and perfectly capable of finding ways of capturing hard-to-get prey.

Smaller shellfish clinging to rocks can be prised off by the otter’s strong arms. The sea otter is fairly strong, but not quite strong enough to defeat the larger abalone, which clings fiercely to rocks. Using rocks as hammers, the otters are capable of dislodging the abalone with several firm strikes from the side.

Underground food such as clams can be obtained by digging. Otters can dig with their forepaws, like dogs, and sometimes use rocks as digging implements. They have a little pouch under their left armpit in which they can store rocks or food, for example, while swimming.

Once the otter has found its food, it carries it to the surface to eat. But not all food can be eaten straightaway. Although sea otters have strong teeth that can bite through crab shells and some shellfish, other sources of prey, e.g. abalones, have to be cracked open first. Individual otters seem to have their own preferences as to which method to use to crack open their prey.

Some otters place a big rock on their chest and then smash their prey on the rock until the shell breaks open. Other otters reverse this operation, placing their prey on the chest and then smashing the rock against it. Some otters use numerous short, rapid strikes, while others administer a few strong blows.

The sea otter’s sense of tidiness can also be observed during eating. Often the otter rolls over in the water to clear its chest of any waste scraps and avoid soiling its coat.

The curious behavior of sea otters in preparing and eating their food is a perfect illustration of isolation and purification.

We hope you like this little story about these cute animals.
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Disclaimer
* For life science research only. Not for use in diagnostic procedures.
+ For general laboratory use.
# CE/for USA for laboratory use
Introduction

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Overview of Nucleic Acid Purification and Isolation  14
Methods described in this manual  16
# Selection Guide

Use this table to select a product according to the type of nucleic acid you wish to purify, then consider the source of the nucleic acid and the scale of the purification.

<table>
<thead>
<tr>
<th>Nucleic Acid Type</th>
<th>Subtype</th>
<th>Origin/Source</th>
<th>Scale</th>
<th>Recommended Product</th>
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<td>Viral DNA</td>
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<td>serum, plasma, whole blood</td>
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Scale:
- micro
- mini
- midi
- maxi
## Selection Guide, continued

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<tr>
<th>Nucleic Acid Type</th>
<th>Origin/Source</th>
<th>Scale</th>
<th>Recommended Product</th>
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<tr>
<td>Total RNA</td>
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<td>formalin-fixed, paraffin-embedded tissue</td>
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<td>animal tissue, stabilized animal tissue, animal cell culture, formalin-fixed, paraffin-embedded tissue</td>
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<td>•</td>
<td>mini Quick Spin Columns</td>
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### Purification Method/Product

**Ultrafiltration**

| High Pure 96 UF Cleanup Kit | PCR products (100 bp to > 10 kb), 20–300 μl | ≥25 μl (>150 bp, ≥40%) 1500 bp, < 20 min | ≥85% (400 bp), ≥60% (600 bp), >20 min | PCR, long-PCR | >200 bp, >100 bp, >100 bp, >20 min | DNA synthesis/primers, restriction enzymes | ddNA synthesis/primers, restriction enzymes | >200 bp, >100 bp, >100 bp, >20 min | ddNA synthesis/primers, restriction enzymes | ddNA synthesis/primers, restriction enzymes | ddNA synthesis/primers, restriction enzymes | ddNA synthesis/primers, restriction enzymes |
|-----------------------------|-----------------------------------------------|---------------------------------------------|------------------------------------------|--------------|-------------------------------------|------------------------------------------|------------------------------------------|-------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|

**Silica Adsorption**

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<th>blood, 200 – 300 μl cultured cells, 10–108 yeast cells, 10–50 mg muscle tissue, 25–50 mg</th>
<th>5–20 μg</th>
<th>20 min</th>
<th>&lt; 20 min</th>
<th>PCR, long-PCR</th>
<th>&gt;200 bp, &gt;100 bp, &gt;100 bp, &gt;20 min</th>
<th>DNA synthesis/primers, restriction enzymes</th>
<th>ddNA synthesis/primers, restriction enzymes</th>
<th>ddNA synthesis/primers, restriction enzymes</th>
<th>ddNA synthesis/primers, restriction enzymes</th>
<th>ddNA synthesis/primers, restriction enzymes</th>
<th>ddNA synthesis/primers, restriction enzymes</th>
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<tr>
<td>High Pure PCR Product Purification Kit</td>
<td>PCR, modifying, labeling, restriction digestion reaction, 100 μl agarose gel slices</td>
<td>≥60% recovery of 5–25 μg DNA</td>
<td>10 min</td>
<td>PCR, long-PCR</td>
<td>&gt;200 bp, &gt;100 bp, &gt;100 bp, &gt;20 min</td>
<td>DNA synthesis/primers, restriction enzymes</td>
<td>ddNA synthesis/primers, restriction enzymes</td>
<td>ddNA synthesis/primers, restriction enzymes</td>
<td>ddNA synthesis/primers, restriction enzymes</td>
<td>ddNA synthesis/primers, restriction enzymes</td>
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<td>DNA synthesis/primers, restriction enzymes</td>
<td>ddNA synthesis/primers, restriction enzymes</td>
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### Recommended Uses

**Purification method/Product Starting material and quantity** | **Yield/recovery** | **Time required** | **PCR/long-PCR** | **ddNA synthesis/primers, restriction enzymes** | **PCR/long-PCR** | **DNA synthesis/primers, restriction enzymes** | **PCR/long-PCR** | **ddNA synthesis/primers, restriction enzymes** | **PCR/long-PCR** | **ddNA synthesis/primers, restriction enzymes** | **PCR/long-PCR** | **ddNA synthesis/primers, restriction enzymes** | **PCR/long-PCR** | **ddNA synthesis/primers, restriction enzymes** |
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<tr>
<td><strong>High Pure 96 UF Cleanup Kit</strong></td>
<td>PCR products (100 bp to &gt; 10 kb), 20–300 μl</td>
<td>≥25 μl (&gt;150 bp, ≥40%) 1500 bp, &lt; 20 min</td>
<td>≥85% (400 bp), ≥60% (600 bp), &gt;20 min</td>
<td>PCR, long-PCR</td>
<td>&gt;200 bp, &gt;100 bp, &gt;100 bp, &gt;20 min</td>
<td>DNA synthesis/primers, restriction enzymes</td>
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<td><strong>High Pure PCR Template Preparation Kit</strong>*</td>
<td>blood, 200 – 300 μl cultured cells, 10–108 yeast cells, 10–50 mg muscle tissue, 25–50 mg</td>
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<tr>
<td><strong>High Pure PCR Product Purification Kit</strong></td>
<td>PCR, modifying, labeling, restriction digestion reaction, 100 μl agarose gel slices</td>
<td>≥60% recovery of 5–25 μg DNA</td>
<td>10 min</td>
<td>PCR, long-PCR</td>
<td>&gt;200 bp, &gt;100 bp, &gt;100 bp, &gt;20 min</td>
<td>DNA synthesis/primers, restriction enzymes</td>
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**In vitro transcription** | | | | | | | | | | | | | | | |
# Product Overview

## Detailed Product Characteristics

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<thead>
<tr>
<th>Purification method/Product</th>
<th>Starting material and quantity</th>
<th>Yield/recovery</th>
<th>Time required</th>
<th>PCR/RT-PCR</th>
<th>DD-PCR</th>
<th>cDNA Synthesis/ primer extension</th>
<th>RE digestion</th>
<th>Southern blotting</th>
<th>Labeling</th>
<th>Northern blotting</th>
<th>RNAase protection assays</th>
<th>Cloning</th>
<th>Sequencing</th>
<th>In vitro transcription</th>
<th>In vivo transcription</th>
<th>Transfection</th>
<th>Microarray Spotting</th>
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<tr>
<td><strong>Solution–based Isolation</strong></td>
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<tr>
<td>DNA Isolation Kit for Cells and Tissues</td>
<td>tissue, 100 mg – 1g cultured cells, 1 x 10^6 – 5 x 10^6 mouse tail, 50 – 400 mg yeast, up to 2 x 10^7 bacteria, up to 1 x 10^11</td>
<td>depending on tissue type: 700 – 2,000 μg/mg cells</td>
<td>25 h, excl. resuspension</td>
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<td>Human whole blood; 10 ml mouse or rat whole blood; 10 ml</td>
<td>350 μg</td>
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<td>TriPure Isolation Reagent</td>
<td>RNA from: liver, spleen, 50 mg – 1 g cultured epithelial cells, 10^6 – 10^7</td>
<td>6 – 10 μg/mg tissue</td>
<td>2.5 h</td>
<td>✔️</td>
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<td>DNA from: liver, kidney, brain, 50 mg – 1 g cultured cells, human, rat, 10^6 – 10^7</td>
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<tr>
<td>Gel filtration</td>
<td>Quick Spin Columns for radiolabeled DNA purification, Sephadex G-25</td>
<td>up to 50 μl labeling mixture</td>
<td>&gt;90%</td>
<td>✔️</td>
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<td>Quick Spin Columns for radiolabeled RNA purification, Sephadex G-20</td>
<td>up to 100 μl labeling mixture</td>
<td>&gt;90%</td>
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<td>Quick Spin Columns for radiolabeled RNA purification, Sephadex G-75</td>
<td>up to 50 μl labeling mixture</td>
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<tr>
<td>mini Quick Spin DNA Columns</td>
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Overview of Nucleic Acid Purification and Isolation

Purification or isolation of nucleic acids is the first step in most molecular biology studies and all recombinant DNA techniques. As a plethora of methods exists for extraction and purification of nucleic acid, researchers usually choose the technique most suited to their:

- Target nucleic acid (ssDNA, dsDNA, total RNA, mRNA, etc.)
- Source organism (mammalian, lower eukaryotes, plants, prokaryotes, viruses, etc.)
- Starting material (whole organ, tissue, cell culture, blood, etc.)
- Desired results (yield, purity, purification time required, etc.)
- Downstream application (PCR, cloning, labeling, blotting, RT-PCR, cDNA synthesis, RNase protection assays, etc.)

Extraction methods

The extraction of nucleic acids from biological material requires cell lysis, inactivation of cellular nucleases, and separation of the desired nucleic acid from cellular debris.

Often, the ideal lysis procedure is a compromise. It must be rigorous enough to fragment the complex starting material (e.g., blood, tissue), yet gentle enough to preserve the target nucleic acid. Common lysis procedures include:

- Mechanical disruption (for example, grinding, hypotonic lysis)
- Chemical treatment (for example, detergent lysis, chaotropic agents, thiol reduction)
- Enzymatic digestion (for example, proteases)

Cell membrane disruption and inactivation of intracellular nucleases may be combined. For instance, a single solution may contain detergents to solubilize cell membranes and strong chaotropic salts to inactivate intracellular enzymes.

After cell lysis and nuclease inactivation, cellular debris may easily be removed by filtration or precipitation.

Purification methods

Methods for purifying nucleic acids from cell extracts are often combinations of extraction/precipitation, chromatography, centrifugation, electrophoresis, and affinity separation.

**Extraction/precipitation**

Solvent extraction is often used to eliminate contaminants from nucleic acids. For example, a combination of phenol and chloroform are frequently used to remove proteins.

Selective precipitation can also purify nucleic acids. For example, high concentrations of salt (“salting out”) or changes in pH can be used to precipitate proteins.

Precipitation may also be used to concentrate nucleic acids. For example, the target nucleic acids are often precipitated with isopropanol or ethanol. If the amount of target nucleic acid is low, an inert carrier (such as glycogen) can be added to the mixture to increase precipitation efficiency.
Chromatography
Chromatography methods may utilize gel filtration, ion exchange, selective adsorption, or affinity binding.

Gel filtration exploits the molecular sieving properties of porous gel particles. A matrix with defined pore size allows smaller molecules to enter the pores by diffusion whereas bigger molecules are excluded from the pores and eluted at the void volume. Thus, molecules are eluted in order of decreasing molecular size.

Ion exchange chromatography depends on an electrostatic interaction between a target molecule and a functional group on the column matrix. The technique allows concentration and separation of molecules from a large volume in a short time. Nucleic acids – highly negatively charged, linear polyanions – can be eluted from ion exchange columns with simple salt buffers.

In adsorption chromatography, nucleic acids adsorb selectively onto silica or glass in the presence of chaotropic salts, while other biological molecules do not. A low salt buffer or water then elutes the nucleic acids, thereby producing a sample that could be used directly in most downstream applications.

Affinity chromatography is a highly specific adaptation of adsorption chromatography. An immobilized ligand recognizes and binds a particular structure on a biomolecule. Washes then remove unbound components (with different structures). Finally, a “competitor molecule” (which also recognizes the immobilized ligand) floods the binding sites on the affinity matrix, releasing the bound biomolecule.

Centrifugation
Selective centrifugation is a powerful purification method. For example, ultracentrifugation in self-forming CsCl gradients at high g-forces has long been used for plasmid purification.

Frequently, centrifugation is combined with another method. For example:

- Spin column chromatography combines gel filtration and centrifugation to purify DNA or RNA from smaller contaminants (salts, nucleotides, etc.), for buffer exchange, or for size selection.
- Some procedures combine selective adsorption on a chromatographic matrix (see above) with centrifugal elution to selectively purify one type of nucleic acid.

Electrophoresis
Nucleic acids may be separated electrophoretically according to their size. This separation is most commonly done on agarose gels. In the presence of ethidium bromide, the separated nucleic acids may be seen under UV light.

Electrophoretic separation is also frequently used to determine size and purity of DNA. For example, after PCR, electrophoresis is used to quickly check product length and purity (absence of byproducts).

Affinity purification
In recent years, more and more purification methods have combined affinity immobilization of nucleic acids with magnetic separation. For instance, poly(A)+ mRNA may be bound to streptavidin-coated magnetic particles by biotin-labeled oligo(dT) and the particle complex removed from the solution (and unbound contaminants) with a magnet. This solid phase technique simplifies nucleic acid purification, since it can replace several centrifugation, organic extraction, and phase separation steps with a single, rapid magnetic separation step.
Methods described in this manual

This manual describes all the products Roche Applied Science currently sells for manual nucleic acid purification and isolation. They are grouped according to the purification methods they use:

- **High Pure Kits and silica adsorption** combine centrifugation, chromatography on glass fiber fleece, and chaotropic salt extraction. These rapid purification kits eliminate traditional solvent extraction, precipitation, and electrophoresis steps.

- **Ion exchange chromatography** uses different conditions for binding and release of nucleic acids. Solutions are just poured or pipetted into the matrix-filled columns which are run by gravity flow. The DNA obtained by this method has a purity comparable to that obtained when purified twice by CsCl gradient centrifugation.

- **Solution-based isolation** uses proprietary cell lysis and extraction methods that are quicker and safer than standard methods. These products can prepare nucleic acids with minimal handling.

- **Affinity purification** exploits the hybridization properties of nucleic acids. These products eliminate time-consuming centrifugation and electrophoresis steps.

- **Gel filtration** relies on “spin columns” that are ready-to-use. These columns take just minutes to separate nucleic acids from salts, unincorporated nucleotides, linkers, or excess primers.

All these products combine proven, reliable nucleic acid purification methods with Roche Applied Science talent for optimization and innovation. Let this manual show you how our line of nucleic acid purification and isolation products can:

- Process more samples in less time
- Minimize nucleic acid loss and degradation
- Improve the performance and reliability of downstream applications
- Increase laboratory efficiency and safety

We've also included in this manual a short overview of our automated nucleic acid isolation system and of our premium products for PCR and RT-PCR. For detailed information please refer to www.roche-applied-science.com.
# High Pure Kits and Silica Adsorption

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Overview of Silica Adsorption

This chapter describes the High Pure kits and other kits which use silica adsorption to quickly and simply purify small amounts of nucleic acid. All of them use methods that:

- Can process multiple samples in minutes, rather than hours or days
- Require less handling of potentially hazardous samples
- Eliminate phenol extraction, precipitation, and other nucleic acid handling steps that can lead to loss or fragmentation of the desired product

For a quick overview of these products, continue reading this article. Or, for detailed information on the product most relevant to your research, turn to the page that describes the product in detail:

<table>
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<th>If you are interested in</th>
<th>For preparing</th>
<th>See page</th>
</tr>
</thead>
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<tr>
<td>High Pure PCR Template Preparation Kit</td>
<td>Genomic DNA from small amounts of whole blood; buffy coat; cultured cells; tissue; mouse tail; gram positive or gram negative bacteria; dried blood spots or paraffin-embedded, fixed tissue sections</td>
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</tr>
<tr>
<td>High Pure PCR Cleanup Micro Kit</td>
<td>Nucleic acids from PCR or other enzymatic reactions or DNA products from agarose gel slices</td>
<td>34</td>
</tr>
<tr>
<td>High Pure PCR Product Purification Kit</td>
<td>Product DNA (a few hundred bp to 50 kb) from 100 μl PCR or other enzymatic reactions, or 100 mg agarose gel slice</td>
<td>42</td>
</tr>
<tr>
<td>High Pure Plasmid Isolation Kit</td>
<td>Purified plasmid DNA from 0.5 – 4.0 ml cultures of <em>E. coli</em></td>
<td>49</td>
</tr>
<tr>
<td>High Pure RNA Isolation Kit</td>
<td>Intact total RNA from small amounts of whole blood, cultured cells, yeast, gram positive or gram negative bacteria</td>
<td>55</td>
</tr>
<tr>
<td>High Pure RNA Tissue Kit</td>
<td>Intact total RNA from tissues</td>
<td>64</td>
</tr>
<tr>
<td>High Pure FFPE RNA Micro Kit</td>
<td>Total RNA from 1 – 10 μm FFPE tissue sections</td>
<td>69</td>
</tr>
<tr>
<td>High Pure RNA Paraffin Kit</td>
<td>Total RNA from fresh-frozen and formalin-fixed, paraffin-embedded tissue sections up to 20 μm</td>
<td>75</td>
</tr>
<tr>
<td>High Pure miRNA Isolation Kit</td>
<td>Tissue, stabilized tissue, cell culture, FFPE tissue sections</td>
<td>84</td>
</tr>
<tr>
<td>High Pure Viral RNA Kit</td>
<td>Intact viral RNA from 200 – 600 μl of serum, plasma, cell culture supernatant, tears, urine, or breast milk</td>
<td>95</td>
</tr>
<tr>
<td>High Pure Viral Nucleic Acid Kit</td>
<td>Total viral nucleic acids (DNA and RNA) from 200 – 600 μl of serum, plasma, whole blood, or cell culture supernatant</td>
<td>100</td>
</tr>
<tr>
<td>High Pure Viral Nucleic Acid Large Volume Kit</td>
<td>Total viral nucleic acids (DNA and RNA) from 1 – 2.5 ml of serum, plasma, whole blood, or cell culture supernatant</td>
<td>105</td>
</tr>
<tr>
<td>Agarose Gel DNA Extraction Kit</td>
<td>Product DNA (0.4 – 100 kb) from 100 – 200 mg agarose gel slice</td>
<td>122</td>
</tr>
<tr>
<td>High Pure 96 UF Cleanup Kit</td>
<td>Product DNA by high throughput ultrafiltration</td>
<td>127</td>
</tr>
</tbody>
</table>
Principle of silica adsorption

All the kits described in this chapter depend on the tendency of nucleic acids to adsorb to silica (glass) in the presence of a chaotropic salt such as sodium iodide (NaI), guanidine thiocyanate or guanidine hydrochloride (Melzak et al., 1996).

This tendency was discovered by Vogelstein and Gillespie (1979) who found that DNA fragments adsorbed to powdered flint glass in the presence of saturated NaI. Later work showed other nucleic acids adsorbed to glass in the presence of other chaotropes, including:

- DNA plasmids (Marko et al., 1982)
- Single-stranded phage nucleic acids (Kristensen et al., 1987; Zimmermann et al., 1989)
- Genomic DNA (Yamada et al., 1990; Zeillenger et al., 1993)
- Total RNA (Yamada et al., 1990)
- Rapid and simple method for purification of nucleic acids (Boom et al., 1990)

Different types of nucleic acid adsorb more or less tightly to glass depending on the ionic strength and the pH of the surrounding solution. A low salt buffer or water is always used to elute the nucleic acid from the glass. In each kit, this method is optimized to prepare a particular type of nucleic acid.

Overview of the procedure

All the High Pure kits use glass fiber fleece immobilized in a special plastic Filter Tube. The glass fleece filter:

- Adsorbs only nucleic acid, ensuring separation of the target molecules from a complex biological mixture of proteins, sugars, lipids, and other components
- Can be inserted into a microcentrifuge tube and processed in a standard tabletop microcentrifuge
- Allows processing of 0.2 – 2.5 ml samples in a series of centrifugation steps
- Is specially constructed to ensure that contaminants suspended in the Wash Buffer are not retained by the filter or transferred to the eluted, purified nucleic acid

Benefit from smart column design

The High Pure Micro Column

- **Achieve high purity.** Novel optimized columns produce highly concentrated (10 μl) eluate and high recovery rates (>80%) of even small DNA and RNA fragments.
- **Up to 10 μg binding capacity** for use in demanding downstream applications.
- **Avoid carryover contamination** using a column design without a cavity for liquid transfer.

The High Pure Mini Column

- **Perform long template applications.** Efficiently purify high molecular weight DNA (30-50 kb).
- **Maximize performance and accuracy in downstream assays.** Achieve high sensitivity and reproducibility in many applications with a binding capacity of up to 100 μg.
- **Obtain accurate results.** Use highly pure, concentrated (50 μl) nucleic acids in real-time PCR and other applications.
In each High Pure kit, the steps are basically the same and require only a few minutes. The nucleic acids prepared with the High Pure kits may be used directly in a variety of down-stream applications.

<table>
<thead>
<tr>
<th>Substance added to serum</th>
<th>Highest concentration tested with no inhibition in PCR after High Pure purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate</td>
<td>30 mg/ml</td>
</tr>
<tr>
<td>EDTA</td>
<td>300 mg/ml</td>
</tr>
<tr>
<td>Heparin</td>
<td>30 U/ml</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>50 mg/ml</td>
</tr>
</tbody>
</table>

Performance of High Pure Nucleic Acid Purification in the removal of different anticoagulants or human hemoglobin. The table indicates the highest concentration tested which showed no inhibition.

Instead of glass fiber fleece (as in the High Pure kits), one can also use silica beads to adsorb DNA.

- The Agarose Gel DNA Extraction Kit starts with an agarose gel slice containing a DNA fragment, then solubilizes that gel to release the DNA into the starting solution.

The kit uses steps similar to those of the High Pure kits to purify the DNA from the starting material. In each, the nucleic acid is adsorbed to silica in the presence of a chaotropic salt, pelleted by centrifugation (while adsorbed to the silica beads), washed extensively to remove contaminants, then released from the beads with a low salt buffer.

The DNA isolated with the kit is pure enough to be used directly in labeling, sequencing, cloning, and other procedures that require concentrated DNA.

References


High Pure PCR Template Preparation Kit

for preparation of up to 100 nucleic acid samples
Cat. No. 11 796 828 001

**Principle**
Cells are lysed during a short incubation with Proteinase K in the presence of a chaotropic salt (guanidine HCl), which immediately inactivates all nucleases. Cellular nucleic acids bind selectively to glass fiber fleece in a special centrifuge tube. The nucleic acids remain bound while a series of rapid “wash-and-spin” steps remove contaminating small molecules. Finally, low salt elution removes the nucleic acids from the glass fiber fleece. The process does not require precipitation, organic solvent extractions, or extensive handling of the nucleic acids.

**Starting material**
- 200 – 300 μl human whole blood (research samples), containing any anticoagulant
- 200 μl buffy coat (research samples)
- 10⁴ – 10⁶ cultured mammalian cells
- 25 – 50 mg mammalian tissue
- 0.2 – 0.5 cm mouse tail (25 – 50 mg)
- 10⁶ yeast cells
- 10⁷ bacteria cells (gram positive or gram negative)
- Paraffin-embedded, fixed tissue sections
- Dried blood spots

**Application**
- Preparation of high molecular weight nucleic acids (30 – 50 kb), which may be used directly in standard PCR, long template PCR, or Southern blots.

**Time required**
- Total time: approx. 20 min (for whole blood or cultured cells)
- Hands-on time: approx. 12 min (for whole blood or cultured cells)

**Results**
- Yield: Variable, depending on sample type (See the table under Part IV of “How to use the kit” in this article).
- Purity: Purified nucleic acid is free of other cellular components and DNA polymerase inhibitors.

⚠️ RNA can be removed from the purified nucleic acids with an optional RNase digestion (see page 24).

**Benefits**
- **Saves time**, because the kit can produce multiple PCR templates in minutes.
- **Improves PCR reproducibility and reliability**, because the kit removes inhibitors that might cause PCR templates to behave unpredictably.
- **Minimizes DNA loss**, because the kit completely removes contaminants without precipitation or other handling steps that can lead to lost or degraded DNA.
- **Ideal for a wide variety of research projects**, because one kit can purify nucleic acids from many sources.
How to use the kit

I. Flow diagram

The chart shows as an example protocol steps for the sample material whole blood.

II. Kit contents

- Tissue Lysis Buffer with 4 M urea (20 ml)
- Binding Buffer with 6 M guanidine HCl (20 ml)
- Proteinase K, lyophilized recombinant
  
  ```
  Dissolve in 4.5 ml PCR grade water before use. Store aliquots at −15 to −25°C.
  ```
- Inhibitor Removal Buffer (33 ml)
  
  ```
  Add 20 ml absolute ethanol before use.
  ```
- Wash Buffer (20 ml)
  
  ```
  Add 80 ml absolute ethanol to Wash Buffer before use.
II. How to use the kit

- Elution Buffer, low salt (10 mM Tris Buffer, pH 8.5; 40 ml)
  - Warm the Elution Buffer to +70°C before use.
- High Pure Filter Tubes (100 tubes)
- Collection Tubes, 2 ml (400 tubes)

III. Additional materials needed

- Absolute ethanol
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile
- PBS buffer (phosphate buffered saline)
- Isopropanol
- Lysozyme (for bacterial DNA preparations)
- Lyticase (for yeast DNA preparations)
- Disposable syringe (for mouse tail DNA preparations)

IV. Typical nucleic acid yield from different organisms (research samples)

<table>
<thead>
<tr>
<th>Starting material</th>
<th>Sample size</th>
<th>Yield (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood, human</td>
<td>200 μl*</td>
<td>3 – 6 (DNA)</td>
</tr>
<tr>
<td>Buffy coat</td>
<td>200 μl*</td>
<td>20 (DNA)</td>
</tr>
<tr>
<td>Cultured cells (K562)</td>
<td>10⁶ cells</td>
<td>15 – 20 (DNA)</td>
</tr>
<tr>
<td>Calf thymus</td>
<td>25 mg</td>
<td>5 – 10 (DNA)</td>
</tr>
<tr>
<td>Mouse tail</td>
<td>0.2 – 0.5 cm (25 – 50 mg)</td>
<td>5 – 10 (DNA)</td>
</tr>
<tr>
<td>Yeast cells</td>
<td>10⁸ cells</td>
<td>10 – 13 (DNA)</td>
</tr>
<tr>
<td>Bacteria cells</td>
<td>10⁶ cells</td>
<td>1 – 3 (total nucleic acids)</td>
</tr>
<tr>
<td>Dried blood spots</td>
<td>9 mm punch-out</td>
<td>Detectable in PCR</td>
</tr>
</tbody>
</table>

* Typical volume is 200 μl; maximum volume is 300 μl. Yields may vary between different blood donors because they may have different amounts of leukocytes.

V. Protocols for preparing total nucleic acids

Va. Isolation of nucleic acids from 200 μl whole blood, 200 μl buffy coat, or 10⁴ – 10⁸ cultured mammalian cells

- If the sample is <200 μl, add PBS to make the total volume 200 μl. If the sample is >200 μl, increase all volumes proportionally in the steps below. Maximum sample volume is 300 μl.

To a sterile 1.5 ml microcentrifuge tube:

- Add 200 μl sample.
- Add 200 μl Binding Buffer.
- Add 40 μl reconstituted Proteinase K solution and mix the contents of the tube immediately.
- Incubate tube for 10 min at +70°C.
After the incubation, mix sample with 100 μl isopropanol.

To transfer the sample to a High Pure Tube:
- Insert one High Pure Filter Tube into one Collection Tube.
- Pipette entire sample into upper buffer reservoir of the Filter Tube.

Insert the entire High Pure Tube assembly into a standard tabletop microcentrifuge, then centrifuge the tube assembly for 1 min at 8000 x g.

After centrifugation:
- Remove the Filter Tube from the Collection Tube and discard the liquid and the Collection Tube.

To remove inhibitors:
- Add 500 μl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube assembly. Repeat the centrifugation step (1 min at 8000 x g) and discard flowthrough and Collection Tube.
- Reinsert the Filter Tube in a new Collection Tube.

To wash the sample:
- Add 500 μl Wash Buffer to the upper reservoir of the Filter Tube.
- Repeat the centrifugation (as in Step 4).

After the first wash:
- Repeat Step 5.
- Repeat the wash step and centrifugation (Step 7). Discard flowthrough.
- Spin the Filter Tube-Collection Tube assembly for 10 s at maximum speed (approx. 13,000 x g) to remove residual Wash Buffer.

Discard the Collection Tube and insert the Filter Tube in a clean, sterile 1.5 ml microcentrifuge tube.

To elute the nucleic acid:
- Add 200 μl of prewarmed (+70°C) Elution Buffer to the Filter Tube.
- Centrifuge the tube assembly for 1 min at 8000 x g.

To increase elution efficiency, either use more Elution Buffer or leave prewarmed Elution Buffer on glass fleece for 5 min before starting the centrifuge.

The microcentrifuge tube now contains the eluted nucleic acids. You may:
- EITHER use an aliquot of the eluted nucleic acids directly in standard or long template PCR.
- OR store the eluted nucleic acids at +2 to +8°C for later analysis.

Optional RNase digestion: If you wish to remove RNA from the template before PCR, add RNase, DNase-free (Cat. No. 11 119 915 001) to the eluted nucleic acids and incubate at +15 to +25°C or +37°C, as appropriate. For example, add 0.5 μl RNase to the nucleic acids from 10⁶ cells and incubate 15 min at +15 to +25°C or +37°C. For nucleic acids from 10⁷ cells, add 1.5 μl RNase and incubate 30 min at +37°C. For nucleic acids from 10⁸ cells, add 16 μl RNase and incubate 30 min at +37°C. After treatment, the RNase can be removed from the DNA with the High Pure PCR Product Purification Kit.
Vb. Isolation of nucleic acids from dried blood spots

For use of blood dried on a filter paper. We recommend the use a 9 mm punch out of a Whatman 903 Specimen Collection Paper

1. To a sterile 1.5 ml microcentrifuge tube:
   - Add 1-3 punches of at least ~3 mm diameter.
   - Add 200 μl Tissue Lysis Buffer
   - Add 40 μl reconstituted Proteinase K solution and mix the contents of the tube immediately.

2. Incubate tube for 1 h at +55°C.
   - Add 200 μl Binding Buffer.
   - Mix immediately and incubate for 10 min at +70°C
   - After the incubation, mix sample with 100 μl isopropanol.

3. To transfer the sample to a High Pure Tube:
   - Insert one High Pure Filter Tube into one Collection Tube.
   - Pipette entire sample into upper buffer reservoir of the Filter Tube.
   - Take care not to block the pipette tip by the card punches.

4. Insert the entire High Pure Tube assembly into a standard tabletop microcentrifuge, then centrifuge the tube assembly for 1 min at 8000 x g.

5. After centrifugation:
   - Remove the Filter Tube from the Collection Tube and discard the liquid and the Collection Tube.

6. To remove inhibitors:
   - Add 500 μl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube assembly. Repeat the centrifugation step (1 min at 8000 x g) and discard flowthrough and Collection Tube.

7. Reinsert the Filter Tube in a new Collection Tube.

8. To wash the sample:
   - Add 500 μl Wash Buffer to the upper reservoir of the Filter Tube.
   - Repeat the centrifugation (as in Step 4).

9. After the first wash:
   - Repeat Step 5.
   - Repeat the wash step and centrifugation (Step 8). Discard flowthrough.

10. Spin the Filter Tube-Collection Tube assembly for 10 s at maximum speed (approx. 13,000 x g) to remove residual Wash Buffer.

11. Discard the Collection Tube and insert the Filter Tube in a clean, sterile 1.5 ml microcentrifuge tube.

12. To elute the nucleic acid:
   - Add 50-100 μl of prewarmed (+70°C) Elution Buffer to the Filter Tube.
   - Centrifuge the tube assembly for 1 min at 8000 x g.
   - To increase elution efficiency, either use more Elution Buffer or leave prewarmed Elution Buffer on glass fleece for 5 min before starting the centrifuge.

13. The microcentrifuge tube now contains the eluted nucleic acids. You may:
   - EITHER use an aliquot of the eluted nucleic acids directly in standard or long template PCR.
   - OR store the eluted nucleic acids at +2 to +8°C for later analysis.
**Vc. Isolation of nucleic acids from 25 – 50 mg mammalian tissue**

1. To a clean, sterile 1.5 ml microcentrifuge tube:
   - Add a 25 – 50 mg tissue sample.
     - To increase the yield of nucleic acids, cut the sample into small pieces with a scalpel.
   - Add 200 μl Tissue Lysis Buffer.
   - Add 40 μl reconstituted Proteinase K solution and mix the contents of the tube immediately.
   - Incubate tube for 1 h at +55°C.

2. After tissue lysis:
   - Add 200 μl Binding Buffer to the tube and mix well.
   - Incubate for 10 min at +70°C.

3. Add 100 μl isopropanol to the tube, then:
   - Mix the contents of the tube.
   - Draw part of the sample into a 1 ml disposable pipette tip.
     - This step draws insoluble tissue segments into the pipette tip and blocks it.
   - Withdraw and discard the pipette tip, carrying the insoluble tissue segments with it.
   - Pipette the remainder of the liquid sample into the upper reservoir of a combined Filter Tube-Collection Tube assembly.
   - Follow Protocol Va page 24, starting at the first centrifugation step (Step 4).

**Vd. Isolation of nucleic acids from 25 – 50 mg mouse tail**

1. To a clean, sterile 1.5 ml microcentrifuge tube:
   - Add pieces from 0.2 – 0.5 cm (25 – 50 mg) mouse tail.
   - Add 200 μl Tissue Lysis Buffer.
   - Add 40 μl reconstituted Proteinase K solution and mix the contents of the tube immediately.
   - Incubate tube for 3 h at +55°C.

2. Use a 1 ml disposable syringe without needle to shear the lysed tail sample:
   - Draw the sample into the syringe and then expel it again.
   - Repeat the above step twice.

3. To the sheared sample:
   - Add 200 μl Binding Buffer.
   - Add 100 μl isopropanol and mix the contents of the tube well.
   - Centrifuge the tube for 5 min at 13,000 x g.

4. After the centrifugation:
   - Pipette the liquid sample into the upper reservoir of a combined Filter Tube-Collection Tube assembly.
   - Follow Protocol Va page 24, starting at the first centrifugation step (Step 4).
Ve. Isolation of nucleic acids from $10^9$ bacteria or $10^8$ yeast cells

1. In a clean, sterile 1.5 ml microcentrifuge tube:
   - Collect the bacteria ($10^9$ cells) or yeast ($10^8$ cells) by low speed centrifugation (3000 x g, 5 min).
   - Resuspend the cell pellet in 200 μl PBS.
   - Does the sample contain bacteria or yeast?
     - If the sample is bacteria, go to Step 2.
     - If the sample is yeast, go to Step 3.

2. To lyse bacterial cells:
   - Add 15 μl lysozyme solution (10 mg/ml in Tris-HCl, pH 8.0).
   - Incubate the tube for 15 min at +37°C.
   - Go to Step 4.

3. To lyse yeast cells:
   - Add 10 μl lyticase solution (0.5 mg/ml).
   - Incubate the tube for 30 min at +30°C.
   - Go to Step 4.

4. To the lysed cells from Step 2 or Step 3:
   - Add 200 μl Tissue Lysis Buffer.
   - Add 40 μl reconstituted Proteinase K solution and mix the contents of the tube immediately.
   - Incubate tube for 10 min at +70°C.

5. Follow Protocol Va page 24, starting at the addition of isopropanol (Step 2).

Gram-positive bacteria generally require special lysis conditions for efficient DNA preparations. Data (see page 31) of cell lysis conditions for lactobacilli and for staphylococci are kindly provided by C. Bunte, J.A. Straub and C. Hertel, University of Hohenheim, Germany.

Vf. Isolation of nucleic acids from a formalin-fixed paraffin-embedded tissue section

[Protocol was kindly provided by T. Fixemer, Universitäts-Kliniken des Saarlandes, Germany]

1. Soak the tissue section in xylene for approx. 30 min, to deparaffinize it.
   - Incubation time depends on the thickness of the section.

2. Incubate the tissue section for 10 s in each of the following:
   - Absolute ethanol (dehydration)
     - The section should turn white after it is transferred to ethanol.
   - 80% ethanol
   - 60% ethanol
   - 40% ethanol
   - Distilled water (rehydration)

3. To prepare the tissue sample:
   - While viewing the section under a microscope, cut the desired tissue area from the rehydrated section with a scalpel.
   - Transfer the sample to a clean, sterile, preweighed 1.5 ml microcentrifuge tube.
   - Determine the weight of the sample.
To the tissue sample (25 – 50 mg):

- Add 200 μl Tissue Lysis Buffer.
- Add 40 μl reconstituted Proteinase K solution and mix the contents of the tube immediately.
- Incubate overnight at +37°C.

**Optimal incubation time will depend on the type of tissue. Determine the incubation time empirically for each new tissue.**

After the first incubation:

- Again, add 20 μl reconstituted Proteinase K solution.
- Incubate at +55°C for 1 – 2 h.

**After this incubation step, no crude tissue particles should be visible.**

To the dissolved tissue sample:

- Add 200 μl Binding Buffer and mix thoroughly.
- Incubate for 10 min at +70°C.

Add 100 μl isopropanol to the tube, then:

- Mix the contents of the tube.
- Use an automatic pipette to draw part of the sample into a 1 ml pipette tip.

**Note: This treatment draws insoluble tissue segments into the pipette tip and blocks it.**

- Withdraw the pipette tip, carrying the insoluble tissue segments with it.
- Pipette the remainder of the liquid sample into the upper reservoir of a combined Filter Tube–Collection Tube assembly.

Follow Protocol Va, page 24, starting at the first centrifugation step (Step 4).

---

**Vg. Isolation of mycobacterial DNA**

(Protocol was kindly provided by B. Leppmeier and U. Reischl, University of Regensburg, Germany)

1. 100 μl decontaminated research sample material (respiratory samples, nonrespiratory samples or sediments from liquid Kirchner media) is centrifuged for 10 min at 14,000 x g. The supernatant is aspirated by pipetting and discarded.

2. Add 200 μl PBS to the pellet and resuspend by vortexing.

3. Add 200 μl Binding Buffer and 40 μl Proteinase K solution to resuspend the pellet. Incubate for 10 min at +70°C.

4. The samples are now alternately incubated for 4 min in a boiling water bath and 2 min in liquid nitrogen. In total the samples are boiled 6 times and frozen 5 times. Then the cooled samples are collected by centrifugation for 1 min at 14,000 x g.

5. Add 100 μl isopropanol and mix by vortexing.

6. Now follow protocol Va, page 24, starting with the transfer of the sample to the Filter Tube assembly (Step 3).

---

**VI. Troubleshooting the High Pure protocols**

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocols, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure PCR Template Preparation Kit, see page 114.
Typical results with the kit

**Experiment 1**

Figure 1: Use of nucleic acids from whole human blood or cultured cells (prepared with the High Pure PCR Template Preparation Kit) for amplification of the single copy human tPA gene. Nucleic acids were prepared from blood or cultured human K562 cells according to Protocol Va, and 250 ng of each preparation were amplified by Expand Long Template PCR. Annealing temperature was +65°C. The PCR cycling conditions and the sequence of the tPA primers were as described in the Expand Long Template PCR package insert. The amplicons obtained were:

- **Lane 2:** 6.3 kb: Obtained from blood, amplified in Expand Long Template PCR buffer 1
- **Lane 3:** 15 kb: Obtained from blood, amplified in Expand Long Template PCR buffer 3
- **Lane 4:** 23 kb: Obtained from blood, amplified in Expand Long Template PCR buffer 3
- **Lane 5:** 28 kb: Obtained from K562 cells, amplified with the Expand 20 kbPLUS System

**Result:** All preparations yielded a distinct specific band of the expected fragment length even as large as 28 kb from genomic DNA prepared with the High Pure PCR Template Preparation Kit.

**Experiment 2**

Figure 2: Use of nucleic acids from mammalian cells or tissue (prepared with the High Pure PCR Template Preparation Kit) for detection of the actin gene on a Southern blot. Nucleic acids were purified from cultured mammalian cells (10^6 K562 cells) by Protocol Va and from calf thymus tissue (25 mg) by Protocol Vb. Aliquots of the preparations were digested with Eco RI, purified (see below), separated electrophoretically, and transferred to a membrane by Southern blotting. The blot was hybridized to a DIG-labeled β-actin antisense RNA probe according to standard procedures. The hybridized bands were detected with an alkaline phosphatase-labeled anti-DIG antibody and visualized chemiluminescently with CSPD substrate. The blot was exposed to X-ray film for 1 h.

- **Lanes 1, 9:** DNA Molecular Weight Marker III
- **Lanes 2, 3:** 1 μg and 3 μg samples of K562 genomic DNA, purified with the High Pure PCR Product Purification Kit after Eco RI digestion
- **Lanes 4 – 6:** 0.3 μg, 1 μg, and 3 μg samples of K562 genomic DNA, purified by traditional methods (phenol/chloroform extraction, ethanol precipitation) after Eco RI digestion
- **Lanes 7, 8:** 0.4 μg and 4 μg samples of calf thymus genomic DNA, purified by traditional methods (phenol/chloroform extraction, ethanol precipitation) after Eco RI digestion

**Result:** All genomic DNA samples isolated with the High Pure PCR Template Preparation Kit were readily and completely cut by Eco RI, were suitable for Southern blot analysis, and contained the expected small and large fragments of the actin gene.
Experiment 3

Figure 3: Use of nucleic acids from paraffin-embedded tissue (prepared with the High Pure PCR Template Preparation Kit) for amplification of the human androgen receptor gene. Nucleic acids were purified from formalin-fixed, paraffin-embedded samples of human prostate tissue by Protocol Ve. An aliquot (10 μl) of each nucleic acid product was used as template for the Expand High Fidelity PCR System (100 μl reaction mixture). The primers were derived from exon 7 of the androgen receptor gene. Aliquots (20 μl) of each amplicon were separated electrophoretically and the gel was stained with ethidium bromide.

Lane 1: Molecular Weight Marker VIII
Lane 2: Androgen receptor amplicon from one tissue section
Lane 3: Androgen receptor amplicon from half a tissue section

Result: The 263 bp androgen receptor amplicon was clearly visible in both tissue samples.

Experiment 4

<table>
<thead>
<tr>
<th></th>
<th>Incubation period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td><strong>L. casei</strong></td>
<td></td>
</tr>
<tr>
<td>Without Mu</td>
<td>15.2 (40.0 %)</td>
</tr>
<tr>
<td>0.25 U/μl Mu</td>
<td>15.3 (40.3 %)</td>
</tr>
<tr>
<td>0.5 U/μl Mu</td>
<td>16.4 (43.2 %)</td>
</tr>
<tr>
<td><strong>L. curvatus</strong></td>
<td></td>
</tr>
<tr>
<td>Without Mu</td>
<td>9.2 (10.6 %)</td>
</tr>
<tr>
<td>0.25 U/μl Mu</td>
<td>25.9 (29.9 %)</td>
</tr>
<tr>
<td>0.5 U/μl Mu</td>
<td>46.0 (53.2 %)</td>
</tr>
<tr>
<td><strong>L. sakei</strong></td>
<td></td>
</tr>
<tr>
<td>Without Mu</td>
<td>13.5 (11.4 %)</td>
</tr>
<tr>
<td>0.25 U/μl Mu</td>
<td>35.0 (29.6 %)</td>
</tr>
<tr>
<td>0.5 U/μl Mu</td>
<td>79.3 (670 %)</td>
</tr>
<tr>
<td><strong>S. aureus</strong></td>
<td></td>
</tr>
<tr>
<td>Without Ly</td>
<td>10.3 (10.8 %)</td>
</tr>
<tr>
<td>10 μg/ml Ly</td>
<td>16.5 (123 %)</td>
</tr>
<tr>
<td>25 μg/ml Ly</td>
<td>16.2 (120 %)</td>
</tr>
</tbody>
</table>

Table 1: Use special lysis conditions for gram-positive bacteria to achieve high DNA concentrations (μg/ml) in eluate. The Lysis Buffer contained 10 mg/ml lysozyme for lactobacilli and 1 mg/ml lysozyme for staphylococci together with the amounts of mutanolysin (MU) or lysostaphin (Ly) shown in the first column. The percentages refer to the DNA yield obtained with the highest mutanolysin or lysostaphin concentration and an incubation period of 30 min.
High Pure PCR Template Preparation Kit

Typical results with the kit

Experiment 5

Figure 4: Agarose gel electrophoresis of the PCR products for *S. aureus* (A) and for various lactobacilli (B)

A:
- Lanes 1 and 12: Molecular Weight Marker 1 kb ladder
- Lane 2: Negative control (without DNA)
- Lanes 3 – 5: *S. aureus*; lysis without lysostaphin, incubation periods 15, 30 and 60 min
- Lanes 6 – 8: *S. aureus*; lysis with 10 μg/ml lysostaphin, incubation periods 15, 30 and 60 min
- Lanes 9 – 11: *S. aureus*; lysis with 25 μg/ml lysostaphin, incubation periods 15, 30 and 60 min

B:
- Lanes 13 and 18: Marker I DNA Hind III
- Lane 14: *L. casei*
- Lane 15: *L. curvatus*
- Lane 16: *L. sakei*
- Lane 17: Negative control (without DNA)

Figure 5: Gel electrophoresis of PCR products and controls.

- **Lane 1**: Smear +++; Specimen sputum; known *M. tuberculosis* infection
- **Lane 2**: Smear –; Specimen sputum
- **Lane 3**: Smear –; Specimen feces
- **Lane 4**: Smear –; Specimen feces; double because of different wash step
- **Lane 5**: Smear +/–; Specimen sputum
- **Lane 6**: Smear +; Specimen BAL
- **Lane 7**: Smear ++; Specimen sputum; COBAS® AMPLICOR® MTB +
- **Lane 8**: Smear +++; Specimen sputum; known *M. avium* infection
- **Lane 9**: Smear +++; Specimen sputum; known *M. avium* infection
- **Lane 10**: Smear +; Specimen sputum
- **Lane 11**: Smear +; Specimen liquid Kirchner media sediment; Smear: acid-fast, rod-shaped
- **Lanes 12 – 22**: Internal controls of samples of lane 1 – 11
- **Lane 23**: Negative control
- **Lane 24**: Positive control *M. tuberculosis* H37
- **Lane 25**: Molecular Weight Marker VIII (Roche Applied Science)

Microbacterial DNA was isolated from clinical research specimen or sediments of liquid cultures by the High Pure PCR Template Preparation Kit. The eluted DNA was analyzed by amplification detection in *M. tuberculosis*-specific PCR (IS6110 target). To control the successful amplification an agarose gel electrophoresis was performed.

**Results:** All internal controls (100 pg *M. tuberculosis* H37 DNA) are positive (lanes 12 – 22, 242 bp amplification product). This indicates the absence of PCR inhibitors within the DNA preparation. Samples of lanes 1, 5, 6, 7, 10 are assessed as positive which is in accordance with the corresponding smear results.
**LightCycler® sample preparation**

The High Pure PCR Template Preparation Kit has been evaluated for LightCycler® sample preparation with whole blood and cultured cells as sample material. Amplification has been performed in LightCycler® capillaries using SYBR Green and Hybridization Probes as the detection formats. The following tables give information about the range of sample volume applied, modifications in the sample preparation procedure and expected results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume/Amount Range</th>
<th>Typical concentration range [ng/μl]</th>
<th>Eluate use in LightCycler® PCR (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood human*</td>
<td>1 – 50 μl **</td>
<td>0.5 – 25</td>
<td>1 – 5 [0.5 ng – 125 ng]</td>
</tr>
<tr>
<td>Cultured cells, K562*</td>
<td>100 – 10^6 cells</td>
<td>0.01 – 10</td>
<td>1 – 5 [0.01 – 50 ng]</td>
</tr>
</tbody>
</table>

* Research samples
** Yield may vary between different blood donors because they may have different amounts of leukocytes

**Procedure modification**

The standard procedure for whole blood and cultured cells has been used for LightCycler® sample preparation except the elution volume was set to 50 μl in order to increase nucleic acid concentration for minute sample amounts. For larger sample amounts the standard elution volume of 200 μl can be applied.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample Material</th>
<th>SYBR Green</th>
<th>HybProbes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>cyclophilin A</td>
<td>blood</td>
<td>0.005 μl</td>
<td>100 μl</td>
</tr>
<tr>
<td>or β-globin</td>
<td>cells</td>
<td>1^a</td>
<td>10^6</td>
</tr>
</tbody>
</table>

The above table shows the minimal and maximal sample amounts which have been purified and used for LightCycler® Amplification with whole blood and cultured cells as sample material. The parameters human cyclophilin A and β-globin have been investigated. All values are for 20 μl LightCycler® Amplification when 5 μl of 50 μl total eluate is applied.

a) The used quantities of eluate correspond to these calculated amounts.
References

High Pure PCR Cleanup Micro Kit

for purification of products from PCR and other reactions
Cat. No. 04 983 955 001 (up to 50 purifications)
Cat. No. 04 983 912 001 (up to 200 purifications)

**Principle**
Nucleic acids bind specifically to the surface of glass fibers in the presence of chaotropic salts. Since the binding process is specific for nucleic acid, the bound material can be separated and purified from impurities by a simple wash step. The Binding Enhancer enables the modification of DNA fragment size exclusions. Small oligonucleotides and dimerized primers from amplification reactions are selectively removed. The nucleic acids elute from the glass fiber fleece in a low-salt buffer or water.

**Starting material**
Samples (up to 100 μl each) could contain:
- Amplified DNA products that are between 50 bp and 5 kb long
- Modified DNA fragments [e.g., DNA processed with restriction enzymes, T4 polymerase or other enzymes] that are between 50 bp and 5 kb long
- Hapten-labeled (e.g., DIG-labeled) or fluorescently labeled DNA fragments
- RNA from *in vitro* transcription reactions
- First and second strand cDNA
- Samples (up to 100 mg) of agarose gel slices

**Application**
Use the High Pure PCR Cleanup Micro Kit to efficiently purify products from PCR and other reactions. The kit eliminates contaminants from amplification reactions (e.g., primers, mineral oil, salts) and can also be used to purify nucleic acids from other modification reactions such as restriction endonuclease digests, alkaline phosphatase treatment, and kinase reactions. In addition, the kit can be utilized to purify cDNA, concentrate dilute nucleic acid solutions, and recover DNA from agarose gel slices. The purified DNA can be used directly in subsequent enzymatic reactions such as labeling, sequencing, cloning, and ligation, as well as for PCR analysis.

<table>
<thead>
<tr>
<th>For 100 μl liquid sample or 100 mg agarose gel slice</th>
<th>200 μl Binding Buffer + 200 μl Binding Enhancer (40%)</th>
<th>300 μl Binding Buffer + 100 μl Binding Enhancer (20%)</th>
<th>400 μl Binding Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purification of liquid sample, 100 μl</td>
<td>Labeling or other reaction products 100 bp to 5 kb +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR products 50 bp to 5 kb</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA fragments for sequencing</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Purification from Agarose Gel, 100 mg</td>
<td>DNA fragments 100 bp to 5 kb +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Removal of low molecular DNA Primer up to 25 bases</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Primer-Dimer up to 70 bp</td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

Nucleic Acid Isolation and Purification Manual
<table>
<thead>
<tr>
<th><strong>Time required</strong></th>
<th>The entire High Pure PCR Cleanup Micro Kit method takes approx. 10 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Results</strong></td>
<td>The amount of DNA recovered is dependent on the amount of DNA applied to the glass fiber fleece, the elution volume, and the length of the amplification/DNA products. When 5 - 25 μg DNA is applied to the kit’s High Pure Micro Filter Tube, approximately 80% of the DNA can be recovered.</td>
</tr>
</tbody>
</table>
| **Benefits**       | • Conserve resources by using one versatile kit that eliminates the need to use several kits from other suppliers.  
• Save time with a simple and rapid protocol that reduces purification time.  
• Obtain purified product in a small elution volume (≤10 μl) for demanding downstream applications.  
• Efficiently remove contaminants and unwanted reaction components.  
• Generate contaminant-free DNA for direct use in cloning, ligation, restriction digests, and other reactions.  
• Selectively isolate specific DNA fragment sizes by using the kit’s binding enhancer to adjust purification stringency. |
How to use the kit

I. Flow diagram

1. Add 100 μl reaction solution
2. Mix well and apply mixture to a High Pure filter tube; centrifuge at 8,000 x g for 30 – 60 s
3. Discard flowthrough
4. Centrifuge at 8,000 x g for 30 – 60 s
5. Discard flowthrough
6. Centrifuge at 8,000 x g for 30 – 60 s
7. Discard flowthrough
8. Centrifuge at max. speed for 1 min
9. Discard collection tube
10. Centrifuge at 8,000 x g for 1 min
11. Purified PCR or other reaction product

Add one of the following*:
- 200 μl Binding Buffer + 200 μl Binding Enhancer, or
- 300 μl Binding Buffer + 100 μl Binding Enhancer, or
- 400 μl Binding Buffer

II. Kit contents

- Binding Buffer 20 ml or 80 ml
- Binding Enhancer 15 ml or 45 ml
- Wash Buffer 10 ml or 2 x 20 ml
- Elution Buffer 40 ml
- High Pure Micro Filter Tubes (50 or 200 tubes)
- Collection Tubes (50 or 200 tubes)

* Please select from the Application Selection Guide of the pack insert.
III. Additional materials needed

The following additional reagents and equipment are required for cleaning up DNA fragments from solutions:

- Absolute ethanol
- Standard tabletop microcentrifuge capable of 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile

*Do not use vessels or pipettes containing polystyrene (PS) when working with the Binding Enhancer (bottle 2).*

The following additional reagents and equipment are required for cleaning up DNA fragments from agarose slices:

- Absolute ethanol
- Agarose
- TAE buffer (40 mM Tris-acetate, 1 mM EDTA) pH 8.4 or TBE buffer (89 mM Tris-borate, 2 mM EDTA) pH 8.4
- Electrophoresis equipment
- Sterile scalpel
- Standard tabletop microcentrifuge capable of 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile

IVa. Purification of PCR Products from Solution

Use the following procedure to remove low molecular weight DNA up to 70 bp from PCR or other reactions. For other applications please refer to Application Selection Guide in the pack insert.

> To process a larger sample (>100 μl), divide it into several 100 μl aliquots and process each as a separate sample.

> Make sure that your sample is in a tube that can hold more than 500 μl. If the sample is > 100 μl, it should be in a 1.5 ml tube.

1. After the PCR is finished, adjust total volume of each sample to 100 μl by adding PCR grade water.
   - To each 100 μl PCR sample, add:
     - 400 μl Binding Buffer
     - Mix sample well by vortexing (e.g., vortex twice, for 4 s each).
     - Centrifuge the mixture briefly.

2. Insert one High Pure Filter Tube into one Collection Tube.
   - Using a pipette, transfer the sample from step 1 to the upper reservoir of the Filter Tube.
   - Centrifuge 30-60 s at 8,000 x g in a standard table top centrifuge at +15 to +25°C.

3. Disconnect the Filter Tube, and discard the flowthrough solution.
   - Reconnect the Filter Tube to the same Collection Tube.

4. Add 400 μl Wash Buffer to the upper reservoir.
   - Centrifuge 30-60 s at 8,000 x g (as above).
High Pure PCR Cleanup Micro Kit
How to use the kit

2

Discard the flowthrough solution.

- Reconnect the Filter Tube to the same Collection Tube.
- Add 300 μl Wash Buffer.
- Centrifuge 30–60 s at 8,000 x g (as above).

! This second (300 μl) wash step ensures optimal purity.

Discard the flowthrough solution.

- Reconnect the Filter Tube to the same Collection Tube.
- Centrifuge 1 min at maximum speed.
- Discard the flowthrough solution and the Collection Tube.
- Connect the Filter Tube to a clean 1.5 ml microcentrifuge tube.

! This step ensures complete removal of Wash Buffer.

Add 10 – 20 μl Elution Buffer to the center of the Filter Tube.

- Centrifuge 1 min at 8,000 x g.

! Do not use water for elution, since alkaline pH is required for optimal yield.

The microcentrifuge tube now contains the purified DNA.

If you plan to determine the $A_{260}$ of the eluted DNA, first centrifuge the eluate for more than 1 min at maximum speed to remove residual glass fibers, which may interfere with the absorbance measurement. Use an aliquot of the supernatant to determine concentration.

Either use the eluted DNA directly or store the eluted DNA at +2 to +8°C or –15 to –25°C for later analysis.

IVb. Purification of DNA Fragments from Agarose Gel

Use the following procedure to purify DNA from a 100 mg agarose gel slice.

1. Isolate DNA band of interest via agarose gel electrophoresis as follows:
   - Load PCR product mixture on a 0.8 - 2% agarose gel.
   - Use 1 x TAE or 1 x TBE as running buffer.
   - Electrophorese until DNA band of interest is separated from adjacent contaminating fragments.

2. Identify bands by staining gel with ethidium bromide or SYBR Green I Nucleic Acid Gel Stain.

   ! Wear gloves; ethidium bromide is a potent carcinogen.

3. Cut desired DNA band from gel using a scalpel or razor blade that has been sterilized with ethanol.

   ! Minimize volume of slice by placing gel on a UV light box (to make the DNA visible) and cutting the smallest possible gel slice that contains the desired DNA.

4. Preweigh an empty, sterile 1.5 ml microcentrifuge tube.

   - Place excised agarose gel slice in the sterile microcentrifuge tube.
   - Determine gel weight by reweighing the tube containing the gel slice and subtracting the weight of the empty tube.

5. To the microcentrifuge tube, add 300 μl Binding Buffer for every 100 mg agarose gel in the tube.
Dissolve agarose gel slice in order to release the DNA.
- Vortex the microcentrifuge tube 15 - 30 s to resuspend the gel slice in the Binding Buffer.
- Incubate the suspension for 10 min at +56°C.
- Vortex the tube briefly every 2 - 3 min during incubation.

After the agarose gel slice is completely dissolved:
- Add 100 μl Binding Enhancer for every 100 mg agarose gel slice in the tube.
- Vortex thoroughly.
- Centrifuge the mixture (dissolved agarose gel slice in Binding Buffer + Binding Enhancer) briefly.

Insert one High Pure Filter Tube into one Collection Tube.
- Using a pipette, transfer the sample from step 7 to the upper reservoir of the Filter Tube.
- Centrifuge 30-60 s at 8000 x g in a standard table top centrifuge at +15 to +25°C.

Follow Protocol IV a above starting at Step 3

IV. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocols, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure PCR Cleanup Micro Kit, see page 115.
Typical results with the kit

Experiment 1

Lane 1: Molecular weight marker VI
Lane 2: 0% Binding Enhancer
Lane 3: 10% Binding Enhancer
Lane 4: 20% Binding Enhancer
Lane 5: 40% Binding Enhancer
Lane 6: PCR without purification
Lane 7: PCR negative control (PCR without template)
Lane 8: Molecular weight marker VI

Figure 6: 1% agarose gel electrophoresis of 341 bp PCR product recovered in the presence of different amounts of Binding Enhancer.

Experiment 2

Lane 1: Competitor Q
Lane 2: Competitor MN
Lane 3: Competitor P
Lane 4: Roche; 0% Binding Enhancer
Lane 5: Roche; 20% Binding Enhancer
Lane 6: Roche; 40% Binding Enhancer
Lane 7: Molecular weight marker VIII, before purification

Figure 7: Electropherogram of the DNA fragment distribution obtained from an Agilent Bioanalyzer run (Agilent, USA).
References

Lassonczyk, N. et al. (2007) Biochemica 3, 18 – 20
Navarro F. et al. (2009) Blood, 114, 2181 – 2192
# High Pure PCR Product Purification Kit

for purification of DNA from PCR reactions

Cat. No. 11 732 668 001 (up to 50 purifications)
Cat. No. 11 732 676 001 (up to 250 purifications)

## Principle

In the presence of chaotropic salt, product DNA binds selectively to glass fiber fleece in a special centrifuge tube. The DNA remains bound while a series of rapid “wash-and-spin” steps remove contaminating small molecules (including small nucleic acids). Finally, low salt elution removes the DNA from the glass fiber fleece. The process does not require DNA precipitation, organic solvent extractions, or extensive handling.

## Starting material

- Research samples (100 μl) may be:
  - DNA products (>100 bp – 50 kb) from PCR
  - Enzymatically labeled, modified, or digested DNA (for example, products from restriction digests, alkaline phosphatase treatments, kinase reactions, or enzymatic labeling reactions)
  - RNA from *in vitro* transcription reactions
  - DNA from a 100 mg agarose gel slice

## Application

- Preparation of concentrated, purified DNA, which may be used directly for labeling, sequencing, cloning and other routine applications.

## Time required

- Total time: approx. 10 min
- Hands-on time: <10 min

## Results

- Yield: Variable, depending on sample volume and DNA size (See the table under Part IV of “How to use the kit” in this article).
- Purity: Purified DNA is free of short DNA (<100 bp), small molecules (for example, mineral oil, primers, salts, unincorporated nucleotides) and proteins (for example, thermostable enzymes).

## Benefits

- **Saves time**, because the kit makes PCR products ready for downstream procedures in less than 10 min.
- **Minimizes DNA loss**, because the kit removes contaminants without precipitation or other handling steps that lead to DNA loss or degradation.
- **Increases lab safety**, because the kit does not use hazardous organic solvents.
- **Accommodates a wide variety of samples**, because the kit can purify DNA from most enzymatic reaction mixtures, DNA from agarose gels, even RNA from *in vitro* transcription reactions.
- **Improves performance of downstream applications**, because the kit removes DNA fragments smaller than 100 bp from the preparation.
How to use the kit

I. Flow diagram

II. Kit contents

- Binding Buffer with guanidine thiocyanate (30 ml or 150 ml)
- Wash Buffer (10 ml or 50 ml)
  
  Add absolute ethanol (40 ml or 200 ml) to Wash Buffer before first use
- Elution Buffer (Tris-HCl buffer, pH 8.5) (40 ml)
- High Pure Filter Tubes (50 or 250 tubes)
- Collection Tubes, 2 ml (50 or 250 tubes)

Both sizes of the kit contain the same components; only the amount of the components in the kit changes.

III. Additional materials needed

- Absolute ethanol
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile
- Agarose
- TAE Buffer (40 mM Tris-acetate, 1 mM EDTA), pH 8.0
- TBE Buffer (89 mM Tris-borate, 2 mM EDTA) pH 7.8
- Electrophoresis equipment
- Sterile scalpel
- Isopropanol
IV. Expected DNA recovery from various amounts and sizes of DNA

<table>
<thead>
<tr>
<th>DNA applied (μg)</th>
<th>Recovery (%)</th>
<th>Fragment length (bp)</th>
<th>Recovery (%)</th>
<th>Elution volume1 (μl)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>77</td>
<td>&lt;100</td>
<td>&lt; 5</td>
<td>50</td>
<td>68</td>
</tr>
<tr>
<td>10</td>
<td>79</td>
<td>375</td>
<td>&gt;95</td>
<td>100</td>
<td>79</td>
</tr>
<tr>
<td>25</td>
<td>80</td>
<td>700</td>
<td>&gt;95</td>
<td>150</td>
<td>80</td>
</tr>
<tr>
<td>50</td>
<td>56</td>
<td>3000</td>
<td>&gt;95</td>
<td>200</td>
<td>80</td>
</tr>
</tbody>
</table>

1 10 μg DNA was used for this experiment.
2 200 μl Elution Buffer was used for this experiment.

V. Protocols for preparing DNA

Va. Purifying amplification products from 100 μl PCR product mix

To process a larger sample (>100 μl), either increase proportionally the amount of Binding Buffer (Step 1) or divide the large sample into several aliquots and process the aliquots as separate samples.

1 After the PCR amplification is complete, adjust the volume in one PCR tube (reaction components + DNA product) to 100 μl, then:
   ▶ Add 500 μl Binding Buffer to the contents (100 μl) of the PCR tube.
   ⚠ You do not need to remove mineral oil or wax from the PCR solution before adding the Binding Buffer.
   ▶ Mix the sample (Binding Buffer + starting PCR solution) well.

2 After mixing the sample:
   ▶ Insert one High Pure Filter Tube into one Collection Tube.
   ▶ Pipette entire sample into upper buffer reservoir of the Filter Tube.

3 Insert the entire High Pure Tube assembly into a standard tabletop microcentrifuge, then centrifuge the tube assembly for 30 s at full speed (approx. 13,000 x g).

4 After the centrifugation:
   ▶ Remove the Filter Tube from the Collection Tube.
   ▶ Discard the flowthrough liquid in the Collection Tube.
   ▶ Reinsert the Filter Tube in the same Collection Tube.

5 To wash the sample:
   ▶ Add 500 μl Wash Buffer to the upper reservoir of the Filter Tube.
   ▶ Repeat the centrifugation (as in Step 3).

6 After the first wash:
   ▶ Discard the flowthrough and recombine the tubes (as in Step 4).
   ▶ Add 200 μl Wash Buffer to the upper reservoir of the Filter Tube.
   ▶ Repeat the centrifugation (as in Step 3).
After the second wash:

- Remove the Filter Tube from the Collection Tube.
- Discard the Collection Tube (with the flowthrough liquid).
- Insert the Filter Tube into a clean, sterile 1.5 ml microcentrifuge tube.

To elute the DNA from the Filter Tube:

- Add 50 – 100 μl Elution Buffer or PCR grade water (pH 8.0 – 8.5) to the upper reservoir of the Filter Tube.
  
  **For optimal recovery of DNA, use 100 μl buffer or water.**

- Centrifuge the tube assembly for 30 s at full speed.

The microcentrifuge tube now contains the eluted DNA. You may:

- EITHER use the eluted DNA directly in such applications as cloning or sequencing
- OR store the eluted DNA at +2 to +8°C for later analysis.

**Vb. Purifying DNA from a 100 mg slice of agarose gel**

1. Purify the DNA of interest electrophoretically as follows:
   - Prepare an agarose gel with Roche Applied Science agarose (MP, LE or MS) and load the DNA of interest on the gel.
   - Use 1 x TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) or 1 x TBE (45 mM Tris-borate, 1 mM EDTA, pH 8.0) as running buffer.
   - Electrophorese until the DNA of interest is well separated from contaminants.

2. After gel electrophoresis, stain the gel with ethidium bromide.

3. Cut the DNA band from the gel with a sharp scalpel or razor blade.

  **Cut the smallest possible gel slice.**

4. Place the agarose gel slice into a sterile 1.5 ml microcentrifuge tube of known weight, then reweigh the tube to determine the weight of the agarose gel.

5. To the microcentrifuge tube, add 300 μl Binding Buffer for every 100 mg agarose gel.

6. To dissolve the agarose gel slice and release the DNA:
   - Vortex the microcentrifuge tube briefly to resuspend the gel slice in the Binding Buffer.
   - Incubate the suspension for 10 min at +56°C.
   - Vortex the tube briefly every 2 – 3 min during the incubation.

7. After the gel is completely dissolved:
   - To the microcentrifuge tube, add 150 μl isopropanol for every 100 mg agarose gel.
   - Vortex thoroughly.

8. After mixing the components of the microcentrifuge tube:
   - Insert one High Pure Filter Tube into one Collection Tube.
   - Pipette the entire contents of the microcentrifuge tube into the upper reservoir of the Filter Tube.

  **If the volume of gel suspension is >700 μl, divide the suspension into two portions and use separate Filter Tubes for each portion.**

9. Follow Protocol Va above, starting at the centrifugation step (Step 3).
Vc. Purifying labeled probes

1. Fill up the labeling reaction to 100 μl with PCR grade water.
2. Add 500 μl Binding Buffer and mix well.
   - It is important that the volume ratio between sample and Binding Buffer is 1:5. When using other sample volumes than 100 μl, adjust the volume of Binding Buffer accordingly.
3. Follow Protocol Vc starting at the sample transfer to the Filter Tube assembly (Step 2).
   - The elution efficiency is increased with higher volume of Elution Buffer applied. At least 68% and 79% recovery are found with 50 and 100 μl Elution Buffer, respectively. Normally, almost quantitative recovery can be found, as can be determined in a direct detection assay.

VI. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocols, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure PCR Product Purification Kit, see page 116.

Typical results with the kit

Experiment 1

![Gel Image]

Figure 8: Comparison of PCR products purified with the High Pure PCR Product Purification Kit and those purified by phenol/chloroform extraction.
Four different long PCR products were generated with the Expand Long Template PCR System, purified by either of two methods, then analyzed electrophoretically. From left to right, the four products shown on the gel are: a 2.9 kb fragment from the p53 gene; 4.8 kb, 6.3 kb, and 9.3 kb fragments from the tissue plasminogen activator (tPA) gene. For each size product, the gel shows:

Lane 1: DNA before purification
Lane 2: DNA after purification by phenol/chloroform extraction
Lane 3: DNA after purification with the High Pure PCR Product Purification Kit

Result: The High Pure Kit removed primers and primer dimers, while recovering 90% of the long PCR products (as calculated by photospectroscopy).
Experiment 2

Preparation of hybridization probes for the analysis of number and expression patterns from sulfurtransferases from Arabidopsis

(kindly provided by J. Papenbrock, P. von Trzebiatowski and A. Schmidt, University of Hannover, Germany)

Background: Sulfurtransferases are a group of enzymes widely distributed in plants, animals, and bacteria. They catalyze the transfer of a sulfane atom from a donor molecule to a thiophilic acceptor substrate.

In a database search several Arabidopsis EST clones with homologies to bacterial and mammalian sulfurtransferases could be identified. One of them was used to isolate a full length clone by screening an Arabidopsis cDNA library. The protein was expressed in E. coli and was determined to be a sulfurtransferase with high specificity for 3-mercapto-pyruvate. However, RNA expression data, protein contents obtained by Western blot analysis and sulfurtransferase enzyme activity measurements using 3-mercaptopyruvate or thiosulfate as substrates did not correlate with each other in Arabidopsis plants in different developmental states. Therefore, we hypothesize the existence of further sulfurtransferases in Arabidopsis. Different EST clones that show homology to sulfurtransferases and also to senescence-associated proteins are initially characterized.

Methods: Genomic Southern blot was performed as previously described. For preparing the hybridization probes cDNAs of interest were separated from the vectors with the respective restriction enzymes (MST1 coding for the mature 3-mercaptopyruvate sulfurtransferase, 950 bp, Kpn I/Pst I; EST 46D8T7, 600 bp, Sal I/Not I). DNA fragments were separated by agarose gel electrophoresis. The gel pieces containing the MST1 or the 46D8T7 fragment were eluted following exactly the inserted recipe for isolating DNA from agarose gels using the High Pure PCR Product Purification Kit. Eluted DNA was used for random prime labeling.

Northern blot was performed as previously described. The PCR probe used in Northern blotting was purified in the following way: The gel piece of interest was excised and the labeled fragment was purified with the High Pure PCR Product Purification Kit according to the inserted recipe for isolating DNA from agarose gels with an elution volume of 100 μl.

Results: Hybridization of restricted genomic Arabidopsis DNA with a digoxigenin-labeled MST1 probe by random priming reveals the existence of at least two, and probably three different sulfurtransferase genes in the Arabidopsis genome (Figure 9, right). The pattern is similar to the hybridization results using the 46D8T7 cDNA but not identical (Figure 9, left).

RNA homologous to the sequence of EST clone 46D8T7 was expressed in very young plants. The expression level decreases drastically and increases continuously during aging (Figure 10). In the plants of a later developmental stage, expression level parallels the expression of SAG 13 clone, the best marker of senescence known so far.
High Pure PCR Product Purification Kit

Typical results with the kit

Figure 9: Southern blot analysis. Genomic DNA was restricted with BamH I, EcoR I, Hind III, and Xba I overnight, transferred to nylon membrane and hybridized with a digoxigenin probe labeled by random prime labeling.

Left: MST1 (3-mercapto-pyruvate sulfurtransferase) cDNA was used as a probe.

Right: cDNA from the EST clone 46D8T7 was labeled.

Figure 10: Northern blot analysis. Total RNA was extracted from Arabidopsis plants of different ages (from the left to the right: harvested after 10, 14, 17, 21, 24, 28, 31, 35, 38, and 42 days), separated under denaturing conditions and transferred to nylon membranes. Equal loading was controlled by ethidium bromide staining. Filters were hybridized with PCR fragments labeled with digoxigenin. PCR-probe (600 bp) used for hybridizing the filter at the top: cDNA from EST clone 46D8T7. PCR-probe (1300 bp) used for hybridizing the filter at the bottom: cDNA from the SAG13 clone (senescence associated gene).

Discussion: The use of digoxigenin-labeled probes leads to very sharp bands in genomic Southern blotting. Purification of digoxigenin-labeled DNA via PCR using the High Pure PCR Product Purification Kit seems to increase the sensitivity of probes and reduce unspecific binding.

References

Dotta, F. et al. (2007) PNAS, 104, 5115 – 5120
High Pure Plasmid Isolation Kit

for purification of plasmid DNA from bacterial cultures
Cat. No. 11 754 777 001 (up to 50 purifications)
Cat. No. 11 754 785 001 (up to 250 purifications)

**Principle**
Alkaline lysis releases plasmid DNA from bacteria and RNase removes all RNA in the lysate. Then, in the presence of a chaotropic salt (guanidine HCl), plasmid DNA binds selectively to glass fiber fleece in a special centrifuge tube. The DNA remains bound while a series of rapid “wash-and-spin” steps remove contaminating bacterial components. Finally, low salt elution removes the DNA from the glass fiber fleece. The process does not require DNA precipitation, organic solvent extractions, or extensive handling of the DNA.

**Starting material**
- 0.5 – 4.0 ml recombinant *E. coli* cultures research samples (at a density of 1.5 – 5.0 A<sub>600</sub> units per ml)

**Application**
- Preparation of up to 15 μg purified plasmid DNA from bacterial cultures, which may be used directly for PCR, cloning, sequencing, *in vitro* transcription, or generation of labeled hybridization probes.

**Time required**
- Total time: approx. 30 min
- Hands-on time: Minimal hands-on time required

**Results**
- Yield: Variable, depending on the *E. coli* strain (See the table under Part IV of “How to use the kit” in this article).
- Purity: Plasmid DNA is ready to use even in demanding down-stream applications.

**Benefits**
- **Saves time**, because the kit can prepare up to 24 plasmid samples in less than 30 min.
- **Minimizes DNA loss**, because the kit removes contaminants without precipitation or other handling steps that can lead to lost or degraded DNA.
- **Increases lab safety**, because the kit does not use hazardous organic reagents such as cesium chloride, phenol, chloroform, or ethidium bromide.
- **Improves reliability and reproducibility of downstream procedures**, because the kit removes RNA and other impurities that might cause the plasmid DNA to behave unpredictably.
How to use the kit

I. Flow diagram

II. Kit contents

- Suspension Buffer (25 or 80 ml)
- RNase A powder (2.5 mg or 8 mg)
  
  \textit{Dissolve RNase powder in Suspension Buffer before use.}

- Lysis Buffer (25 or 80 ml)
- Binding Buffer with guanidine HCl (25 or 100 ml)

Wash Buffer I containing guanidine HCl (33 or 100 ml); (optional; please see step 7 of protocol)

\textit{Add absolute ethanol (20 ml or 60 ml) to Wash Buffer before use}
How to use the kit

- Wash Buffer II (10 ml or 50 ml)
  - Add absolute ethanol (40 ml or 200 ml) to Wash Buffer before use
- Elution Buffer (Tris-HCl Buffer, pH 8.5) (40 ml)
- High Pure Filter Tubes (50 or 250 tubes)
- Collection Tubes, 2 ml (50 or 250 tubes)

Both sizes of the kit contain the same components; only the amount (values in parentheses) of the components in the kit changes.

III. Additional materials needed

- Absolute ethanol
- Centrifuge tubes and centrifuge for harvesting up to 4 ml bacterial culture
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile

IV. Typical DNA recovery from various E. coli strains/pUC 19

<table>
<thead>
<tr>
<th>E. coli host strain/density</th>
<th>0.5 ml</th>
<th>1.0 ml</th>
<th>2.0 ml</th>
<th>4.0 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>XL 1 blue (3.6 A&lt;sub&gt;600&lt;/sub&gt;/ml)</td>
<td>4.9 μg</td>
<td>8.6 μg</td>
<td>11.8 μg</td>
<td>14.6 μg</td>
</tr>
<tr>
<td>DH 5 α (1.5 A&lt;sub&gt;600&lt;/sub&gt;/ml)</td>
<td>0.9 μg</td>
<td>1.7 μg</td>
<td>3.3 μg</td>
<td>6.2 μg</td>
</tr>
<tr>
<td>HB 101 (4.7 A&lt;sub&gt;600&lt;/sub&gt;/ml)</td>
<td>1.8 μg</td>
<td>3.5 μg</td>
<td>5.9 μg</td>
<td>8.2 μg</td>
</tr>
</tbody>
</table>

V. Protocol for preparing DNA from 0.5 – 4.0 ml of E. coli cell culture

1. Place Binding Buffer on ice.
2. Prepare the starting material:
   - Pellet the bacterial cells from 0.5 – 4.0 ml of E. coli culture.
     - The cells should have a density of 1.5 – 5.0 A<sub>600</sub> units per ml.
   - Discard the supernatant.
   - Add 250 μl Suspension Buffer + RNase to the centrifuge tube containing the bacterial pellet.
   - Resuspend the bacterial pellet and mix well.
3. Treat the resuspended bacterial pellet as follows:
   - Add 250 μl Lysis Buffer.
   - Mix gently by inverting the tube 3 to 6 times.
     - To avoid shearing genomic DNA, do not vortex!
   - Incubate for 5 min at +15 to +25°C.
     - Do not incubate for more than 5 min!
Treat the lysed solution as follows:

1. Add 350 μl chilled Binding Buffer.
2. Mix gently by inverting the tube 3 to 6 times.
3. Incubate on ice for 5 min.

   The solution should become cloudy and a flocculent precipitate should form.

Centrifuge for 10 min at approx. 13,000 x g (full speed) in a standard tabletop microcentrifuge.

After centrifugation:

1. Insert one High Pure Filter Tube into one Collection Tube.
2. Transfer entire supernatant from Step 5 into upper buffer reservoir of the Filter Tube.
3. Insert the entire High Pure Tube assembly into a standard tabletop microcentrifuge.
4. Centrifuge for 30 – 60 s at full speed.

Does the E. coli strain have high nuclease activity?

If no, go to Step 8.

   Example: XL 1 blue and DH 5α do not have high nuclease activity and do not require the optional wash with Wash Buffer I.

If yes, continue this step with the optional wash.

   Example: HB 101 and JM strains have high nuclease activity and require the optional wash with Wash Buffer I.

   (Optional) To eliminate high nuclease activity from the preparation:

1. Add 500 μl of Wash Buffer I to the upper reservoir of the Filter Tube.
2. Centrifuge for 30 – 60 s at full speed and discard the flowthrough.

To wash the cells:

1. Add 700 μl Wash Buffer II to the upper reservoir of the Filter Tube.
2. Centrifuge for 30 – 60 s at full speed and discard the flowthrough.

After discarding the flowthrough liquid:

1. Centrifuge the entire High Pure Tube assembly for an additional 30 to 60 s to remove residual Wash Buffer.
2. Discard the Collection Tube.

To elute the DNA:

1. Insert the Filter Tube into a clean, sterile 1.5 ml microcentrifuge tube.
2. Add 100 μl Elution Buffer or PCR grade water (pH adjusted to 8.0 – 8.5) to the upper reservoir of the Filter Tube.
3. Centrifuge the tube assembly for 30 – 60 s at full speed.

The microcentrifuge tube now contains the eluted plasmid DNA. You may:

1. EITHER use the eluted DNA directly in such applications as cloning or sequencing
2. OR store the eluted DNA at +2 to +8°C or −15 to −25°C for later analysis.
VI. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocol, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure Plasmid Isolation Kit, see page 116.

Typical results with the kit

Figure 11: Automated sequencing of plasmid DNA purified with the High Pure Plasmid Isolation Kit. A derivative of pUC18 containing a 3.3 kb insert was isolated from E. coli XL1 blue (1.5 ml culture) according to the protocol given in this article. Isolated plasmid DNA (250 ng) was sequenced with fluorescent-labeled sequencing primer under the following cycle sequencing conditions: initial denaturation, 2 min at +95°C; then, repeated cycles of denaturation (30 s, +95°C), primer annealing (30 s, +60°C), and extension/termination (60 s, +70°C). Aliquots (2 μl) of the sequencing mixture were applied to a 4.3% PAA gel (66 cm plate). The sequence was analyzed with a LI-COR Automated DNA Sequencer (Model 4000 S) in the autosequencing mode.

Result: More than 700 nucleotides can be read with High Pure Plasmid Isolation Kit prepared sequencing template on a LI-COR model 4000 S automated sequencing system using automated base calling and the autostop function.
References

High Pure RNA Isolation Kit

for isolation of total RNA from up to 50 samples
Cat. No. 11 828 665 001

Principle
A single reagent lyses the sample and inactivates RNase. In the presence of a chaotropic salt (guanidine HCl), the released total RNA binds selectively to glass fiber fleece in a special centrifuge tube. The RNA remains bound while a DNase treatment and a series of rapid “wash-and-spin” steps remove DNA and contaminating small molecules. Finally, low salt elution removes the RNA from the glass fiber fleece. The process does not require precipitation, organic solvent extractions, or extensive handling of the RNA.

Starting material
- $10^6$ cultured mammalian cells (research samples)
- 200 – 500 μl human whole blood (research samples), containing anticoagulant
- $10^8$ yeast (mid- to late-log phase, $A_{600} < 2.0$)
- $10^9$ gram positive or gram negative bacteria

Application
- Preparation of intact total RNA, which may be used directly in RT-PCR, Differential Display RT-PCR (DDRT-PCR), Northern blotting, primer extension assays, RACE (rapid amplification of cDNA ends), cDNA library construction, *in vitro* translation, or nuclease protection assays.

RNA prepared from human whole blood is suitable for RT-PCR only.

Time required
- Total time: approx. 25 min (+ sample pretreatment, if needed)
- Hands-on time: <10 min (+ sample pretreatment, if needed)

Results
- Yield: Variable, depending on sample type (See the table under Part IV of “How to use the kit” in this article).
- Purity: Purified RNA is free of DNA, protein, salts, and other cellular components.

Benefits
- **Saves time**, because the kit can produce multiple RNA samples in minutes.
- **Ideal for a wide variety of samples**, because one kit can purify RNA from many sources.
- **Minimizes RNA loss**, because the kit removes contaminants without time-consuming precipitation or solvent extraction.
- **Increases lab safety**, because the kit does not use hazardous organic solvents.
How to use the kit

I. Flow diagram

II. Kit contents

- Lysis/Binding Buffer containing guanidine HCl and Triton X-100 (25 ml)
- DNase I, lyophilized (10 kU)
  
  ⚠️ Dissolve DNase I in 0.55 ml Elution Buffer and store in aliquots at –15 to –25°C. A 0.11 ml aliquot is enough to process 10 samples.
- DNase Incubation Buffer (10 ml)
- Wash Buffer I containing guanidine HCl (33 ml)
  
  ⚠️ Add 20 ml absolute ethanol to Wash Buffer I before use.
- Wash Buffer II (10 ml)
  - Add 40 ml absolute ethanol to Wash Buffer II before use.
- Elution Buffer (30 ml)
- High Pure Filter Tubes (50 tubes)
- Collection Tubes, 2 ml (50 tubes)

III. Additional materials needed
- Absolute ethanol
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile
- Phosphate buffered saline (PBS), sterile
- Red Blood Cell Lysis Buffer (for human blood)
- Lysozyme, 50 mg/ml (for bacteria)
- 10 mM Tris-HCl, pH 8.0, sterile (for bacteria)
- Lyticase, 0.5 mg/ml (for yeast)
- Standard tabletop centrifuge capable of 3000 x g centrifugal force (for yeast or bacteria)

IV. Typical RNA yield from different organisms (research samples)

<table>
<thead>
<tr>
<th>Starting material</th>
<th>Sample size</th>
<th>Average RNA yield (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured cells (K 562)</td>
<td>10⁶ cells</td>
<td>15</td>
</tr>
<tr>
<td>Whole blood, human</td>
<td>200 – 500 μl</td>
<td>10 RT-PCR</td>
</tr>
<tr>
<td>Yeast (S. cerevisiae)</td>
<td>10⁷ cells</td>
<td>20</td>
</tr>
<tr>
<td>Bacteria (E. coli)</td>
<td>10⁷ cells</td>
<td>50</td>
</tr>
<tr>
<td>Bacteria (B. subtilis)</td>
<td>10⁷ cells</td>
<td>35</td>
</tr>
</tbody>
</table>
V. Protocols for preparing total RNA

Be careful to avoid RNase contamination throughout these protocols. For details, see the package insert provided with the kit.

Va. Isolation of total RNA from 10⁶ cultured mammalian cells

This protocol may also be used to isolate RNA from small blood volumes (<200 μl), as an alternative to Protocol Vb.

1. Harvest about 10⁶ cultured mammalian cells and resuspend in 200 μl PBS.
2. Add 400 μl Lysis/Binding Buffer to the resuspended cells. Mix the contents of the tube well.
3. To transfer the sample to a High Pure Tube:
   - Insert one High Pure Filter Tube into one Collection Tube.
   - Pipette entire sample into upper buffer reservoir of the Filter Tube (max. 700 μl).
4. Insert the entire High Pure Tube assembly into a standard tabletop microcentrifuge, then:
   - Centrifuge the tube assembly for 15 s at approx. 8000 x g.
   - Separate the two tubes and discard the liquid in the Collection Tube.
   - Reinsert the Filter Tube in the same Collection Tube.
5. In a separate, sterile tube, mix 100 μl DNase solution (90 μl DNase Incubation Buffer + 10 μl reconstituted DNase I) for each sample. Then:
   - Add 100 μl DNase solution to the upper reservoir of the Filter Tube.
   - Incubate the Filter Tube for 15 min at +15 to +25°C.
6. After the DNase incubation:
   - Add 500 μl Wash Buffer I to the upper reservoir of the Filter Tube.
   - Repeat the centrifugation (as in Step 4).
7. After the first wash:
   - Add 500 μl Wash Buffer II to the upper reservoir of the Filter Tube.
   - Repeat the centrifugation (as in Step 4).
8. After the second wash:
   - Add 200 μl Wash Buffer II to the upper reservoir of the Filter Tube.
   - Centrifuge the tube assembly for 2 min at maximum speed (approx. 13,000 x g) to remove any residual Wash Buffer.
9. Discard the Collection Tube and insert the Filter Tube in a clean, sterile 1.5 ml microcentrifuge tube.
10. To elute the RNA:
    - Add 50 – 100 μl Elution Buffer to the Filter Tube.
    - Centrifuge the tube assembly for 1 min at 8000 x g.
    - The microcentrifuge tube now contains the eluted total RNA, which may be used directly in a variety of procedures.
**Vb. Isolation of total RNA from 500 μl human blood (research samples)**

**General notes:**
- The blood should be fresh and at +15 to +25°C. It should have been collected in a tube containing sodium EDTA or any other anticoagulant.
- This protocol may be used to prepare RNA from 200 – 500 μl blood.

1. **Prepare sample tubes:**
   - Warm Red Blood Cell Lysis Buffer to +15 to +25°C.
   - For each sample, add 1 ml Red Blood Cell Lysis Buffer to a sterile 1.5 ml microcentrifuge tube.

2. **To each microcentrifuge tube:**
   - Add 500 μl human whole blood.
   - Cap and mix by inversion.
   - *Do not vortex tubes.*

3. **Mix the tubes by:**
   - EITHER placing the microcentrifuge tube on a rocking platform or gyratory shaker for 10 min.
   - OR inverting the sample at regular intervals by hand, for a total of 10 min.

4. **Centrifuge the tube at 500 x g for 5 min in a microcentrifuge.**

5. **With a sterile pipette, carefully remove and discard the clear, red supernatant.**

6. **For each tube:**
   - Add 1 ml Red Blood Cell Lysis Buffer to the white pellet, then cap the tube.
   - “Flick” the tube (with fingers) until the pellet is completely resuspended.
   - *Do not vortex the tube.*

7. **Centrifuge the tube at 500 x g for 3 min.**

8. **With a sterile pipette, carefully remove and discard both the supernatant and the red ring of blood cell debris that forms around the outer surface of the white blood cell pellet.**

9. **The white blood cell pellet is the starting material for RNA isolation. Thus:**
   - Resuspend the white pellet in 200 μl sterile PBS.

10. **Follow Protocol Va above, starting with the addition of Lysis/Binding Buffer (Step 2).**

---

**Alternatively blood stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow can be used**

1. **Mix 0.5 ml whole blood with 1 ml reagent.**
   - This mixture is stable for at least one week at +2 to +8°C or a month at –15 to –25°C.

2. **900 μl lysate (corresponding to 300 μl blood) is transferred to the Filter Tube and centrifuged for 1 min at 8000 x g**
   - *Remove any residual lysis reagent by a second centrifugation step.*
Follow protocol Va starting with the DNase incubation (Step 5).

### Vc. Isolation of total RNA from $10^8$ yeast (S. cerevisiae)

- **Use fresh yeast that were harvested during mid- or late-log phase ($A_{600} < 2.0$).**

1. For each sample ($10^8$ yeast):
   - Collect the yeast by centrifugation at 2000 x $g$ for 5 min in a standard tabletop centrifuge.
   - Resuspend the pellet in 200 μl of PBS and transfer the suspension to a sterile 1.5 ml microcentrifuge tube.

2. Add 10 μl lyticase solution (0.5 mg/ml) to each microcentrifuge tube. Incubate the tube for 15 min at +30°C.

3. Follow Protocol Va above, starting with the Lysis/Binding Buffer step (Step 2).

### Vd. Isolation of total RNA from $10^9$ bacteria (gram positive or gram negative)

1. For each sample ($10^9$ bacteria):
   - Collect the bacteria by centrifugation at approximately 2000 x $g$ for 5 min in a standard tabletop centrifuge.
   - Resuspend the pellet in 200 μl of 10 mM Tris-HCl, pH 8.0, and transfer the suspension to a sterile 1.5 ml microcentrifuge tube.

2. Add 4 μl lysozyme solution (50 mg/ml in Tris-HCl, pH 8.0) to each microcentrifuge tube. Incubate the tube for 10 min at +37°C.

3. Add 400 μl Lysis/Binding Buffer to the tube and mix well.

4. To transfer the sample to a High Pure Tube:
   - Insert one High Pure Filter Tube into one Collection Tube.
   - Pipette entire sample into upper buffer reservoir of the Filter Tube.


6. Prepare DNase solution as in Step 5, Protocol Va. After centrifugation:
   - Add 100 μl DNase solution to the upper reservoir of the Filter Tube.
   - Incubate the Filter Tube for 60 min at +15 to +25°C.

7. Follow Protocol Va above, starting with the first wash (Step 6).

### VI. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocols, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure RNA Isolation Kit, see page 117.
Typical results with the kit

Experiment 1

Figure 12: Effect of DNase treatment on mammalian RT-PCR templates prepared with the High Pure RNA Isolation Kit. RNA was isolated from six identical samples (each containing 10^6 K562 human lymphocyte cells). Four samples were treated with the DNase solution as described in Protocol Va above. For 2 samples, the DNase step was omitted. 10 μl of each sample was used in a first strand cDNA reaction (20 μl total volume) with M-MuLV Reverse Transcriptase and oligo(dT) (as primer). From this first strand cDNA reaction, 10 μl were transferred to a PCR (Expand High Fidelity system) and amplified with a primer pair specific for the GAPDH gene.

Left panel:
Lane 1: MWM VI
Lanes 2 – 7: The RT-PCR produced a specific 983 bp amplification product.

Right panel:
Lane 1: MWM III
Lanes 2 – 7: In a control PCR, the samples were processed as for RT-PCR, except the reverse transcriptase in the first strand cDNA reaction was omitted.

Result: The samples not incubated with DNase gave an amplification product in the PCR control, indicating the presence of some residual genomic DNA (right panel, lanes 6 and 7), samples that were treated with DNase did not give any amplification product (right panel, lanes 2 – 5) in the control.

Experiment 2

Figure 13: Northern analysis of RNA prepared with the High Pure RNA Isolation Kit and a competitor's kit. RNA was isolated from 10^6 K562 human lymphocyte cells with each kit, and the yields determined spectrophotometrically. The indicated amount of each RNA sample was loaded on a denaturing formaldehyde gel, separated electrophoretically, and transferred to a positively charged nylon membrane (Roche Applied Science) by capillary blotting. The blot was hybridized with a digoxigenin-labeled actin RNA probe (Roche Applied Science). Hybridized probe was detected immunochemically and visualized with CSPD chemiluminescence substrate.

Result: A specific signal of the expected size was obtained in all samples, but the specific actin RNA bands in the aliquots prepared with the Roche Applied Science kit were more concentrated and contained less background.
Experiment 3

Analysis with the LightCycler® Instrument of cDNA synthesis derived from total RNA isolated according to the alternative blood protocol forming a lysate with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow (HP-VB) and of a white blood cell lysate (HP-WBC).

Figure 14: Mammaglobin

Figure 15: PBGD

Figure 16: High Pure total RNA Preparation from human whole blood (research samples). The porphobilinogen deaminase (PBGD) mRNA reaction product serves as control for RT-PCR performance and as reference for relative quantification.

10 μl Blood = 30 μl Lysate
20 μl Blood = 60 μl Lysate
50 μl Blood = 150 μl Lysate
100 μl Blood = 300 μl Lysate
200 μl Blood = 600 μl Lysate
300 μl Blood = 900 μl Lysate
Reference

Taguchi F. et al. (2010) Microbiology, 156, 72 – 80
High Pure RNA Tissue Kit

for up to 50 isolations of total RNA from tissue
Cat. No. 12 033 674 001

Principle
Isolating intact RNA is a prerequisite for the analysis of gene expression. Frequently applied techniques like Reverse Transcriptase-PCR (RT-PCR), Northern blotting, and RNase protection require the use of intact undegraded RNA. Tissue samples are disrupted and homogenized in the presence of a strong denaturing buffer containing guanidine hydrochloride to instantaneously inactivate RNases, and to ensure isolation of intact RNA. After adding ethanol RNA binds selectively to a glass fiber fleece in the presence of a chaotropic salt (guanidine HCl). Residual contaminating DNA is digested by DNase I, applied directly on the glass fiber fleece. During a series of rapid "wash-and-spin" steps to remove contaminating cellular components the RNA remains bound to the glass fiber fleece. Finally, low salt elution removes the nucleic acids from the glass fiber. The process does not require RNA precipitation, organic solvent extractions, or extensive handling of the RNA.

Starting material
- Tissue (research samples, e.g., mouse liver, spleen, lung, heart)
- 1 – 10 mg (for mortar/pestle disruption) or
- 1 – 25 mg (for rotor-stator homogenization)

Application
- The kit prepares intact total RNA from tissue samples for direct use as template for RT-PCR or Northern blotting.

Time required
- Total time: approx. 30 min with rotor-stator homogenization. Add additional time when using alternative disruption methods.

Results
- Yield: 0.3 – 3 μg total RNA per mg tissue depending on tissue type.
- Purified RNA is free of DNA, nucleases and all cellular and sample components that interfere with RT-PCR.
- The absence of contaminating DNA is examined by a PCR without a preceding RT-reaction; no amplification product is obtained.

Benefits
- Toxic materials are avoided, no CsCl gradient centrifugation and no extraction with organic solvents is necessary.
- Eluates are ready-to-use, no alcohol precipitation has to be done.
How to use the kit

I. Flow diagram

Lysate supernatant (tissue homogenized in Lysis/Binding Buffer) → Add 0.5 volumes ethanol abs.

Mix and apply lysate (max. 700 μl at a time) to a High Pure Filter Tube Assembly, centrifuge at 13,000 x g for 30 s (repeat if the lysate volume is more than 700 μl)

Discard flowthrough → Incubate at +15 to +25°C for 15 min

Add 80 μl DNase Incubation Buffer/10 μl DNase working solution → Add 500 μl Wash Buffer I

Centrifuge at 8000 x g for 15 s → Add 500 μl Wash Buffer II

Centrifuge at 8000 x g for 15 s → Add 300 μl Wash Buffer II

Centrifuge at 13,000 x g for 2 min → Add 100 μl Elution Buffer

Centrifuge at 8000 x g for 1 min → Pure total RNA

II. Kit contents

- Lysis/Binding Buffer (25 ml)
- DNase I, lyophilizate (10 kU)
- DNase Incubation Buffer (10 ml)
- Wash Buffer I (33 ml)
  - Add 20 ml absolute ethanol before use
- Wash Buffer II (10 ml)
  - Add 40 ml absolute ethanol before use
- Elution Buffer (30 ml)
- High Pure Filter Tubes, (50 tubes)
- Collection Tubes, 2 ml (50 tubes)
III. Additional materials needed

- Absolute ethanol
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile
- Mortar and pestle or Rotor-Stator Homogenizer (e.g., Ultra Turrax)

IV. Typical RNA yield from different type of mouse tissue

The yield of total RNA depends on the starting material and varies depending on the amount of tissue used and the kind of disruption method applied. The yield with mouse muscle tissue could not be determined spectroscopically, but isolated RNA resulted in a specific RT-PCR signal.

<table>
<thead>
<tr>
<th>type of mouse tissue</th>
<th>Yield [μg/mg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>liver</td>
<td>0.5 – 2.8</td>
</tr>
<tr>
<td>kidney</td>
<td>0.5 – 1.0</td>
</tr>
<tr>
<td>spleen</td>
<td>0.5 – 3.0</td>
</tr>
<tr>
<td>lung</td>
<td>0.3 – 0.5</td>
</tr>
<tr>
<td>heart</td>
<td>0.3</td>
</tr>
<tr>
<td>muscle</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

V. Isolation procedure

After you have prepared the working solutions and performed the disruption and homogenization of starting material you can start with the isolation procedure.

**Standard reaction**

1. Depending on the disruption and homogenization method, add one of the following to a nuclease-free 1.5 ml microcentrifuge tube:
   - Add 400 μl Lysis/Binding Buffer and the appropriate amount of frozen tissue (max. 20 – 25 mg); disrupt and homogenize the tissue using a rotor-stator homogenizer.
   - alternative:
     - Add 400 μl Lysis/Binding Buffer and the appropriate amount of tissue-powder (grinded with a mortar and pestle) and pass this lysate 5 – 10 times through a 20-gauge needle fitted to a syringe. For optimal yield do not exceed 10 mg tissue.

2. Centrifuge lysate for 2 min at maximum speed in a microcentrifuge and use only the collected supernatant for subsequent steps.

3. Add 200 μl absolute ethanol to the lysate supernatant and mix well.

4. Combine the High Pure Filter Tube and the Collection Tube and pipette the entire sample in the upper reservoir.

5. Centrifuge for 30 s at maximum speed (13,000 x g) in a standard table top microcentrifuge.

   After this centrifugation step, the glass fleece must be dry; if it looks wet, the centrifugation time must be increased.

   Discard the flowthrough and reassemble the Filter Tube and the used Collection Tube.

6. Into a sterile 1.5 ml reaction tube, pipette 90 μl DNase Incubation Buffer, add 10 μl DNase I working solution, mix. Pipette the solution in the upper reservoir of the Filter Tube. Incubate for 15 min at +15 to +25°C.
Typical results with the kit

Comparison of different lysis/homogenization procedures

Experiment 1

Figure 17: Mouse liver was homogenized with various procedures and RNA purified using the standard protocol of the High Pure RNA Tissue Kit. RT was performed with primers for a region of the GADH mRNA.

Lane 1: Ultra Turrax;
Yield: 1.9 μg/mg tissue; OD_{260}/OD_{280} = 2.0
Lane 2: Disposable plastic pestle, motor driven;
Yield: 3.0 μg/mg tissue; OD_{260}/OD_{280} = 2.0
Lane 3: Mortar + pestle/20G needle;
Yield: 1.5 μg/mg tissue; OD_{260}/OD_{280} = 2.0
Lane 4: Disposable plastic pestle, manual; 20 G needle;
Yield: 1.8 μg/μg tissue; OD_{260}/OD_{280} = 2.0
Lane 5: Disposable plastic pestle, manual;
Yield: 3.4 μg/mg tissue; OD_{260}/OD_{280} = 2.0
Lane 6: Bead-Vortex homogenization;
Yield: 3.0 μg/mg tissue; OD_{260}/OD_{280} = 2.0
Lane 7: MWM
Experiment 2

<table>
<thead>
<tr>
<th>Platinum-resistant ovarian cancer samples</th>
<th>Platinum-sensitive ovarian cancer samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>RNA-Integrity-Number</td>
</tr>
<tr>
<td>1</td>
<td>8.6</td>
</tr>
<tr>
<td>2</td>
<td>8.0</td>
</tr>
<tr>
<td>3</td>
<td>8.2</td>
</tr>
<tr>
<td>4</td>
<td>8.7</td>
</tr>
<tr>
<td>5</td>
<td>8.5</td>
</tr>
<tr>
<td>6</td>
<td>8.2</td>
</tr>
<tr>
<td>7</td>
<td>8.8</td>
</tr>
<tr>
<td>8</td>
<td>8.1</td>
</tr>
<tr>
<td>9</td>
<td>9.1</td>
</tr>
<tr>
<td>10</td>
<td>9.0</td>
</tr>
</tbody>
</table>

Figure 18: Measurement of RNA quality ranking using the Agilent Bioanalyzer results in a RNA Integrity Number (RIN) between 10 (highest) and 1 (lowest). Panel A shows the measured RNA-Integrity-Numbers in the 10 platinum-sensitive and 10 platinum-resistant ovarian cancer tissues (research samples). Panels B and C show an electropherogram overlay of several platinum-resistant (B) and platinum-sensitive (C) ovarian tumor samples. The first big peak at 40 sec marks 18S rRNA, the second peak at 47 sec 28S rRNA; these signals reflect the good quality of total RNA obtained from the fresh frozen ovarian tissue samples.

Reference

High Pure FFPE RNA Micro Kit

for isolation of total RNA from formalin-fixed, paraffin-embedded tissue samples
Cat. No. 04 823 125 001 (up to 50 isolations)

**Principle**
To prepare tissue sections for RNA isolation, fixation reagents must be removed from the samples; after deparaffinization, the sections are ready to be processed with the High Pure FFPE RNA Micro Kit.

The deparaffinized tissue samples are disrupted and homogenized during incubation with Proteinase K and chaotropic salt. The homogenate is then applied to the glass fiber fleece in a High Pure Micro Filter Tube.

Under the buffer conditions used in the procedure, all nucleic acids (NA) bind specifically to the glass fleece in the High Pure tube, while contaminating substances (salts, proteins, and other tissue contaminants) do not. DNA is removed from the filter by digestion with DNase I. A series of rapid “wash-and-spin” steps remove the DNA fragments, along with other contaminating substances. Finally, the purified RNA is eluted from the column in low-salt buffer.

**Starting material**
- Amount: 1 – 10 μm sections
- Type: formalin-fixed, paraffin-embedded (FFPE) tissue
- Source: typical FFPE mammalian tissue (e.g., from colon, breast, liver, kidney, or spleen of mammal species incl. human research samples)

*Section thickness as well as yield and quality of the isolated RNA are strongly related to type of tissue, age of sample as well as fixation protocol used.*

**Application**
The High Pure FFPE RNA Micro Kit is designed for the isolation of total RNA from formalin-fixed, paraffin-embedded tissue samples for use in RT-PCR. The quality of RNA from paraffin-sections achieved with the kit is suitable for the relative quantification of mRNA with RT-PCR especially on the LightCycler® 2.0 System.

**Time required**
- Total time required is approx. 60 min (without Proteinase K incubation)

**Results**
The High Pure FFPE RNA Micro Kit is optimal for FFPE sample material as shown by the high recovery of even small RNA fragments. Average yields are 1.5 to 3.5 μg / 5 μm section DNA-free RNA.

**Benefits**
- **Saves time** with a simple and rapid protocol.
- **Obtain a highly concentrated eluate** (10 μl) and recovery (>80%).
- **Isolate DNA-free RNA**, ideal for use in qualitative and relative quantitative RT-PCR.
- **Efficiently isolate RNA** – even small RNA fragments.
- **Generate high-quality template RNA** that show excellent performance and linearity in RT-PCR.
- **Rely on the innovative column design** to efficiently recover contamination-free RNA.
How to use the kit

I. Flow diagram

1. Place deparaffinized section in a reaction tube
   - Vortex 3 x 4 s and spin down
   - Vortex 3 x 4 s, spin down and incubate for 3 h at +55°C
   - Vortex 3 x 4 s, spin down, and pipette the lysate into the upper reservoir of a High Pure Micro Filter Tube assembly. Centrifuge at 8,000 x g for 30 s
   - Discard flowthrough
   - Centrifuge 1 min at max. speed to dry filter
   - Incubate for 15 min at +15 to +25°C
   - Centrifuge at 8,000 x g for 15 s
   - Discard flowthrough
   - Centrifuge at 8,000 x g for 15 s
   - Discard flowthrough
   - Centrifuge at 8,000 x g for 15 s
   - Discard collection tube
   - Centrifuge for 2 min at max. speed to dry filter.
   - Place the Filter Tube in a fresh 1.5 ml microcentrifuge tube.
   - Incubate for 1 min at +15 to +25°C. Centrifuge at 8,000 x g for 1 min
   - Reload the Eluate, incubate for 1 min at +15 to +25°C. Centrifuge at 8,000 x g for 1 min
   - Pure total RNA

2. Add 60 μl Tissue Lysis Buffer and 10 μl 10% SDS
3. Add 30 μl Proteinase K
4. Add 200 μl Binding Buffer and 200 μl ethanol, abs.
5. Add 30 μl DNase Solution (3 μl DNase Incubation Buffer and 27 μl DNase)
6. Add 300 μl Wash Buffer I
7. Add 300 μl Wash Buffer II
8. Add 200 μl Wash Buffer II
9. Add 300 μl Wash Buffer II
10. Add 20 μl Elution Buffer
II. Kit contents

- Tissue Lysis Buffer, 20 ml
- Proteinase K (recombinant, PCR grade), lyoph., 100 mg
- Binding Buffer, 80 ml
- Wash Buffer I, 33 ml
- Wash Buffer II, 10 ml
- DNase I, lyoph., 4 kU
- DNase Incubation Buffer, 10x conc., 1 ml
- Elution Buffer, 30 ml
- High Pure Micro Filter Tubes (containing glass fiber fleece), 5 x 10 tubes
- Collection Tubes, 2 x 50 tubes

III. Additional materials needed

- Absolute ethanol
- Ethanol 70%
- Hemo-De or Xylene
- SDS 10%
- Microcentrifuge tubes, 1.5 ml/2.0 ml, sterile
- Standard tabletop microcentrifuge capable of 13,000 x g centrifugal force

IV. Protocol for the Isolation of RNA from Formalin-fixed, Paraffin-Embedded Tissue

IVa. Deparaffinization procedure for one 1–10 μm section of formalin-fixed, paraffin-embedded tissue in a 1.5 ml reaction tube.

1. To one 1 – 10 μm section in a 1.5 ml reaction tube add 800 μl Hemo-De (or Xylene), at least vortex for 4 s three times, incubate for 2 min, vortex at least for 4 s three times again and incubate for 5 min. Centrifuge for 2 min at maximum speed (12,000 – 14,000 x g) and discard supernatant by aspiration.

   After this step directly cap the tubes to avoid tissue sections from drying.

   If the tissue appears to be floating in the Xylene, spin for a further 2 min.

2. Repeat Step 1.

3. Add 800 μl ethanol abs., flick the tube to dislodge the pellet and vortex for 4 s three times.
   Centrifuge for 2 min at maximum speed (12,000 – 14,000 x g) and discard supernatant by aspiration.

4. Add 800 μl 70% ethanol flick the tube to dislodge the pellet and vortex for 4 s three times.
   Centrifuge for 2 min at maximum speed and discard supernatant by aspiration.

5. After removal of 70% ethanol, respin the tube for 10 – 20 s and carefully remove the residual fluid with a fine bore pipette.
   Incubate the open tubes in a heating block for 5 – 15 min at +55°C to air dry the tissue pellet.
   Proceed with step 1 of the RNA isolation protocol (IVc)
IVb. Deparaffinization procedure for one 1 – 10 μm section of formalin-fixed, paraffin-embedded tissue on a microscope slide.

1. Place the slide in a Hemo-De (or Xylene) bath and incubate for 10 min.
2. Tap off excess liquid and place the slide into ethanol abs. for 10 min.
3. Change bath and incubate the slide for a further 10 min in ethanol abs.
4. Scratch the deparaffinized section from the slide by using a sterile single-use scalpel and place it in a 1.5 ml reaction tube.

- To avoid scattering of the tissue, scratch the section from the microscope slide before it has dried.
- Dry the tissue for 10 min at +55°C.
- Proceed with step 1 of the RNA isolation protocol(IVc).

IVc. RNA Isolation Protocol from a 1-10 μm section of formalin-fixed, paraffin-embedded tissue.

- If necessary, multiple preparations can be pooled after step 4.

1. To one tissue pellet (deparaffinized as described above) add 60 μl Tissue Lysis Buffer and 10 μl 10% SDS
   - Vortex 3 x 4 s, spin down and add 30 μl Proteinase K working solution.
   - Vortex 3 x 4 s, spin down and incubate for 3 h at +55°C.
2. Add 200 μl Binding Buffer and 200 μl ethanol abs.
   - Vortex 3 x 4 s and spin down
3. Combine the High Pure filter tube with a collection tube and pipette the lysate into the upper reservoir.
   - Centrifuge for 30 s at 8,000 x g in a microcentrifuge and discard the flowthrough.
   - Steps 3-4 can be repeated, in order to load the column with additional sample material (do not overload the column).
4. Centrifuge for 1 min at max. speed to dry filter.
5. Add 30 μl DNase Solution (3 μl DNase Incubation Buffer and 27 μl DNase), Incubate for 15 min at +15 to +25°C.
6. Add 300 μl Wash Buffer I working solution to the upper reservoir.
   - Centrifuge for 15 s at 8000 x g, discard the flowthrough.
7. Add 300 μl Wash Buffer II working solution
   - Centrifuge for 15 s at 8000 x g, discard the flowthrough.
8. Add 200 μl Wash Buffer II working solution
   - Centrifuge for 15 s at 8000 x g, discard the collection tube.
9. Place the High Pure Micro filter tube in a fresh collection tube and centrifuge for 2 min at maximum speed.
10. Place the High Pure Micro filter tube in a fresh 1.5 ml reaction tube, add 20 μl Elution Buffer and incubate for 1 min at +15 to +25°C.
    - Centrifuge for 1 min at 8000 x g.
11. Reload the eluate; incubate for 1 min at +15 to +25°C. Centrifuge at 8000 x g for 1 min.
The microcentrifuge tube now contains the eluted RNA. Either use 0.5 – 5 μl of the eluted RNA directly in RT-PCR or store the eluted RNA at ~80°C for later analysis.

Before photometric determination of the RNA concentration, centrifuge the eluate for 2 min at maximum speed and transfer supernatant to a fresh 1.5 ml reaction tube without disturbing glass fibers at the bottom of the original tube.

V. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocols, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure FFPE RNA Micro Kit, see page 119.

Typical results with the kit

Figure 19: Fragment-length distribution of isolated RNA. RNA was isolated from a 5 μm section of an FFPE breast tumor research sample, using either the High Pure FFPE RNA Micro Kit or a kit from another manufacturer (Supplier X). The size distribution of the recovered RNA fragments was determined on an electropherogram (Bioanalyzer, Agilent).

Result: The High Pure FFPE RNA Micro Kit recovers optimal amounts of the different RNA fragments in the sample, even the small ones.

Figure 20: Performance of isolated RNA in RT-PCR. RNA was isolated from a 5 μm section of an FFPE breast tumor research sample, using either the High Pure FFPE RNA Micro Kit or a kit from another manufacturer (Supplier X). The isolated RNA samples were serially diluted and used as templates in separate RT-PCRs. A β-2microtubulin-specific amplification was performed, using the LightCycler® 1.5 Instrument and LightCycler® RNA Amplification Kit SYBR Green I.

Result: Template RNA isolated with the High Pure FFPE RNA Micro Kit performs well in the RT-PCR, giving linear results (based on the consistent slope obtained with serial dilutions) and high sensitivity (based on the early crossing points observed).
References

High Pure RNA Paraffin Kit

for up to 100 isolations of total RNA from fresh-frozen and formalin-fixed, paraffin-embedded tissue

Cat. No. 03 270 289 001

**Principle**

Tissue samples are disrupted and homogenized during an overnight incubation with Proteinase K (paraffin samples) or by using a suitable tissue homogenizer (fresh-frozen tissue). Nucleic acids bind in the presence of a chaotropic salt specifically to the surface of glass fibers prepacked in the High Pure Filter Tube. The binding process is specific for nucleic acids in general, but the binding conditions are optimized for RNA. Bound RNA is purified in a series of rapid „wash-and-spin“ steps to remove contaminating cellular components. Residual DNA is digested by incubation with DNase I. A second incubation step with Proteinase K improves the purity of RNA. Finally, low salt elution removes the nucleic acids from the glass fiber. The process does not require RNA precipitation, organic solvent extractions, or extensive handling of the RNA.

**Starting material**

- 10 – 13 μg sections from formalin-fixed, paraffin-embedded tissue (research samples)
- 10 – 30 mg fresh-frozen solid tissue (research samples)
- 3 – 5 μm tissue section from fresh-frozen tissue (research samples)

**Application**

- The kit is designed for the isolation of total RNA for use in RT-PCR. The quality of the RNA from paraffin sections is suitable for relative quantification of mRNA with RT-PCR especially on the LightCycler® 2.0 Instrument.

**Time required**

- Total time approx. 2 h without overnight incubation

**Results**

- 0.3 – 1.5 μg total RNA per 5 μg formalin-fixed, paraffin-embedded tissue section depending on tissue type and section size.
- 2 – 6 μg total RNA per 20 mg fresh-frozen solid tissue depending on tissue type.
- 0.5 – 5 μg total RNA per 3 x 5 μg section from fresh solid tissue depending on tissue type and section size.

**Benefits**

- **Toxic materials are avoided**, no organic solvent extraction is necessary.
- **Eluates are ready to use**, no RNA precipitation required.
- **Improved purity** due to extensive purification method.
How to use the kit

I. Flow diagram

1. Place deparaffinized section in a reaction tube
2. Add 100 μl Tissue Lysis Buffer, 16 μl 10% SDS and 40 μl Proteinase K
3. Vortex briefly in several intervals and incubate overnight at +55°C
4. Mix gently, and pipette the lysate into the upper reservoir of a High Pure Filter Tube assembly. Repeat the step for remaining lysate
5. Centrifuge for 30 s at 8000 x g. Centrifuge at max. speed to dry filter fleece
6. Discard flowthrough
7. Add 325 μl Binding Buffer and 325 μl ethanol, abs.
8. Centrifuge for 15 s at 8000 x g
9. Discard flowthrough
10. Add 500 μl Wash Buffer I
11. Centrifuge for 15 s at 8000 x g
12. Discard flowthrough
13. Add 500 μl Wash Buffer II
14. Centrifuge for 15 s at 8000 x g
15. Discard flowthrough
16. Add 300 μl Wash Buffer II
17. Centrifuge for 15 s at 8000 x g
18. Discard flowthrough
19. Centrifuge for 2 min at max. speed. Place Filter Tube in fresh 1.5 ml microcentrifuge tube. Centrifuge 1 min at 8000 x g
20. Discard Collection Tube
21. Mix and incubate for 45 min at +37°C
22. Vortex briefly and incubate for 1 h at +55°C

Continued on next page
II. Kit contents

- Tissue Lysis Buffer (20 ml)
- Proteinase K (2 x 100 mg)
- Binding Buffer (2 x 80 ml)
- Wash Buffer I (100 ml)
  
  🚫 Add 60 ml absolute ethanol before first use
- Wash Buffer II (50 ml)
  
  🚫 Add 200 ml absolute ethanol before first use
- DNase I (4 kU)
- DNase Incubation Buffer, 10 x conc. (2 x 1 ml)
High Pure RNA Paraffin Kit

How to use the kit

- Elution Buffer (2 x 30 ml)
- High Pure Filter Tubes (200 tubes)
- Collection Tubes, 2 ml (200 tubes)

III. Additional materials needed

- Hemo-De or Xylene
- Ethanol, absolute
- SDS 10%
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, sterile
- Homogenization device

IV. Typical downstream application

- The kit is optimized for the isolation of RNA from paraffin-embedded tissue fixed with the neutral buffered 10% formalin. Different fixation procedures of the tissue may lead to variations regarding RNA yields.
- RNA suitable for RT-PCR has been isolated by this kit from archived paraffin-tissue up to 15 years old.
- 10 μl of the RNA eluate is used in one-step RT-PCR and specific primers for β2M-gene. In the following LightCycler® PCR accomplished with LightCycler® RNA Amplification Kit SYBR Green I and specific primers for β2M the expected amplification signal is obtained at a cp-value <24.
- Absence of contaminating genomic DNA is examined by a RT-PCR without addition of reverse transcriptase; no amplification product is obtained.
## V. Isolation procedure

### Va. RNA isolation from 1 μm x 5 – 10 μm section of formalin-fixed, paraffin-embedded tissue

If necessary 3 preparations can be pooled after step 4.

1. To one tissue pellet (deparaffinized as described in package insert) add 100 μl Tissue Lysis Buffer, 16 μl 10% SDS and 40 μl Proteinase K. Vortex briefly in several intervals and incubate overnight at +55°C.

2. Add 325 μl Binding Buffer and 325 μl ethanol abs. Mix gently by pipetting up and down.

3. Combine the High Pure Filter Tube and the Collection Tube and pipette the lysate into the upper reservoir.

4. Centrifuge for 30 s at 8000 x g in a microcentrifuge and discard the flowthrough.

5. Repeat the centrifugation at maximum speed in order to dry the filter fleece completely.

6. Add 500 μl Wash Buffer I working solution to the upper reservoir. Centrifuge for 15 s at 8000 x g, discard the flowthrough.

7. Add 500 μl Wash Buffer II. Centrifuge for 15 s at 8000 x g, discard the flowthrough.

8. Add 300 μl Wash Buffer II. Centrifuge for 15 s at 8000 x g, discard the flowthrough.

9. Centrifuge the High Pure Filter Tube for 2 min at maximum speed.

10. Place the High Pure Filter Tube in a fresh 1.5 ml reaction tube, add 90 μl Elution Buffer. Centrifuge for 1 min at 8000 x g.

11. Add 10 μl DNase Incubation Buffer, 10x and 1.0 μl DNase I working solution to the eluate and mix. Incubate for 45 min at +37°C.

12. Add 20 μl Tissue Lysis Buffer, 18 μl 10% SDS and 40 μl Proteinase K. Vortex briefly. Incubate for 1 h at +55°C.

13. Add 325 μl Binding Buffer and 325 μl ethanol abs. Mix and pipette into a fresh High Pure Filter Tube with Collection Tube.

14. Centrifuge for 30 s at 8000 x g in a microcentrifuge and discard the flowthrough.

15. Repeat the centrifugation at maximum speed in order to dry the filter fleece completely.

16. Add 500 μl Wash Buffer I to the upper reservoir. Centrifuge for 15 s at 8000 x g, discard the flowthrough.

17. Add 500 μl Wash Buffer II. Centrifuge for 15 s at 8000 x g, discard the flowthrough.

18. Add 300 μl Wash Buffer II. Centrifuge for 15 s at 8000 x g, discard the flowthrough.

19. Centrifuge the High Pure Filter Tube for 2 min at maximum speed.

20. Place the High Pure Filter Tube in a fresh 1.5 ml reaction tube. Add 50 μl Elution Buffer, incubate for 1 min at +15 to +25°C. Centrifuge for 1 min at 8000 x g to collect the eluted RNA.

21. Use RNA directly for cDNA synthesis or store at -80°C.
How to use the kit

Vb. RNA isolation from 20 – 30 mg of fresh frozen tissue

Before you begin efficient disruption and homogenization of the sample material is essential for intracellular RNA isolation procedures from tissues. The complete disruption of cell walls and plasma membranes of cells and organelles is absolutely required to release all the RNA contained in the sample. Incomplete disruption results in significantly reduced yields of RNA. Homogenization is necessary to reduce the viscosity of the cell lysates produced by disruption. Homogenization shears the high molecular weight genomic DNA and other high molecular weight cellular components to create a homogeneous lysate. Incomplete homogenization results in significantly reduced yields.

1. Homogenize 20 – 30 mg of fresh-frozen tissue with suitable method.
2. Centrifuge the lysate for 2 min at maximum speed in a microcentrifuge. Transfer the supernatant to a fresh 2 ml reaction tube.
3. Add 1 ml Binding Buffer and 600 μl ethanol abs. Mix gently by pipetting up and down.
4. Combine the High Pure Filter Tube and the Collection Tube and pipette half of the volume of the lysate into the upper reservoir.
5. Centrifuge for 30 s at 8000 x g in a microcentrifuge and discard the flowthrough.
6. Pipette the rest of the lysate into the same High Pure Filter Tube and centrifuge for 30 s as described in step 5.
7. Repeat the centrifugation at maximum speed in order to dry the filter fleece completely.
8. Add 500 μl Wash Buffer I to the upper reservoir. Centrifuge for 15 s at 8000 x g, discard the flowthrough.
9. Add 500 μl Wash Buffer II. Centrifuge for 15 s at 8000 x g, discard the flowthrough.
10. Add 300 μl Wash Buffer II. Centrifuge for 15 s at 8000 x g, discard the flowthrough.
11. Centrifuge for 2 min at maximum speed.
12. Place The High Pure Filter Tube in a fresh 1.5 ml reaction tube. Add 90 μl Elution Buffer. Centrifuge for 1 min at 8000 x g.
13. Add 10 μl DNase Incubation Buffer, 10x, 1.0 μl DNase I working solution to the eluate and mix. Incubate for 45 min at +37°C.
14. Add 20 μl Tissue Lysis Buffer 18 μl 10% SDS and 40 μl Proteinase K. Vortex briefly. Incubate for 1 h at +55°C.
15. Add 325 μl Binding Buffer and 325 μl ethanol abs., mix by pipetting. Pipette into a fresh High Pure Filter Tube with Collection Tube.
16. Centrifuge for 30 s at 8000 x g in a microcentrifuge and discard the flowthrough.
17. Repeat the centrifugation at maximum speed in order to dry the filter fleece completely.
18. Add 500 μl Wash Buffer I to the upper reservoir. Centrifuge for 15 s at 8000 x g, discard the flowthrough.
19. Add 500 μl Wash Buffer II. Centrifuge for 15 s at 8000 x g, discard the flowthrough.
20. Add 300 μl Wash Buffer II. Centrifuge for 15 s at 8000 x g, discard the flowthrough.
21. Centrifuge the High Pure Filter Tube for 2 min at maximum speed.
22. Place the High Pure Filter Tube in a fresh 1.5 ml reaction tube. Add 70 μl Elution Buffer. Centrifuge for 1 min at 8000 x g to collect the eluted RNA.
23. Use RNA directly for cDNA synthesis or store at −80°C.
## How to use the kit

### Vc. RNA isolation from 3 x 5 μm sections of fresh-frozen tissue

1. To 3 x 5 μm sections of fresh-frozen tissue in a 1.5 ml reaction tube add 100 μl Tissue Lysis Buffer 16 μl 10% SDS and 40 μl Proteinase K. Vortex in several intervals and incubate overnight at +55°C.

2. Add 325 μl Binding Buffer and 325 μl ethanol abs. Mix gently by pipetting up and down.

3. Combine the High Pure Filter Tube and the Collection Tube and pipette the lysate into the upper reservoir.

4. Centrifuge for 30 s at 8000 x g in a microcentrifuge and discard the flowthrough.

5. Repeat the centrifugation at maximum speed in order to dry the filter fleece completely.

6. Add 500 μl Wash Buffer I to upper reservoir. Centrifuge for 15 s at 8000 x g, discard the flowthrough.

7. Add 500 μl Wash Buffer II. Centrifuge for 15 s at 8000 x g, discard the flowthrough.

8. Add 300 μl Wash Buffer II. Centrifuge for 15 s at 8000 x g, discard the flowthrough.

9. Centrifuge the High Pure Filter Tube for 2 min at maximum speed.

10. Place the High Pure Filter Tube in a fresh 1.5 ml reaction tube. Add 90 μl Elution Buffer. Centrifuge for 1 min at 8000 x g.

11. Add 10 μl DNase Incubation Buffer, 1.0 μl DNase I working solution to the eluate and mix. Incubate for 45 min at +37°C.

12. Add 20 μl Tissue Lysis Buffer, 18 μl 10% SDS and 40 μl Proteinase K. Vortex briefly. Incubate for 1 h at +55°C.

13. Add 325 μl Binding Buffer and 325 μl ethanol abs., mix and pipette into a fresh High Pure Filter Tube with Collection Tube.

14. Centrifuge for 30 s at 8000 x g in a microcentrifuge and discard the flowthrough.

15. Repeat the centrifugation at maximum speed in order to dry the filter fleece completely.

16. Add 500 μl Wash Buffer I to the upper reservoir. Centrifuge for 15 s at 8000 x g, discard the flowthrough.

17. Add 500 μl Wash Buffer II. Centrifuge for 15 s at 8000 x g, discard the flowthrough.

18. Add 300 μl Wash Buffer II. Centrifuge for 15 s at 8000 x g, discard the flowthrough.

19. Centrifuge the High Pure Filter Tube for 2 min at maximum speed.

20. Place the High Pure Filter Tube in a fresh 1.5 ml reaction tube. Add 70 μl Elution Buffer. Centrifuge for 1 min at 8000 x g to collect the eluted RNA.

21. Use RNA directly for cDNA synthesis or store at –80°C.

### VI. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure Kits. For details on how to troubleshoot the above protocol, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure RNA Paraffin Kit, see page 120.
Typical results with the kit

Experiment 1

Figure 21: Linear measuring range of TP, DPD TS and G6PDH
LightCycler® System of RNA dilution series for TP, DPD, TS and G6PDH (1.5 μg – 2.5 ng RNA). RNA was isolated from formalin-fixed, paraffin embedded colon sections using the High Pure RNA Paraffin Kit. Each RNA concentration was 5 times in an independent reaction reverse transcribed, using specific primers. PCR assays were performed with the LightCycler® TP/DPD/TS mRNA Quantification Kits. The determined dynamic range covers 2 log stages of the analyzed RNA concentrations.

Experiment 2

Figure 22: Specificity control of the LightCycler® PCR products
After RT-PCR reaction, LightCycler® (LC) PCR products of TP, DPD, TS and G6PDH, were analyzed on a 3% agarose gel. RT controls for each target were performed and negative in subsequent LC PCR assays (data not shown).
- Lanes 1 – 3: LightCycler® PCR product of DPD (dihydropyrimidine dehydrogenase)
- Lanes 4 – 6: LightCycler® PCR product of TP (thymidine phosphorylase)
- Lanes 7 – 9: LightCycler® PCR product of TS (thymidylate synthetase)
- Lanes 10 – 12: LightCycler® PCR product of G6PDH (glucose-6-phosphate dehydrogenase)
- Lane 13: water control
Reference

Evtimova, V. et al. (2006) Biochemica 1
Ploughman M. et al. (2009) Stroke, 40, 1490 – 1495
# High Pure miRNA Isolation Kit

for low to medium miRNA isolation  
Cat. No. 05 080 576 001 (up to 50 miRNA isolations)

## Principle
In the presence of the chaotropic salt guanidine thiocyanate, RNA binds selectively to special glass fibers pre-packed in the High Pure Filter Tube. Bound RNA is purified in a series of rapid wash-and-spin steps to remove contaminating salts, proteins and other cellular impurities and then eluted using a low salt solution. This simple method eliminates the need for organic solvent extractions and RNA precipitation, allowing for rapid purification of many samples simultaneously. By lowering the concentration of binding enhancer during the binding step in the two-column protocol the small RNA containing miRNA passes the first column unbound. When the concentration of Binding enhancer is increased the small RNA fraction can be bound to a second High Pure Filter Tube.

## Starting material
- Animal tissue 1 – 50 mg either fresh deep frozen or stored in “RNAlater®”
- Animal cell culture up to $10^6$ cells
- Paraffin-embedded tissue 5 – 10 μm sections
- Liquid samples up to 150 μl: from cytoplasmic extracts or enzymatic reactions (e.g., DNase digestion, RNA labeling, RNA ligations, *In vitro* transcriptions)

## Application
The kit is designed for the isolation of small RNA (e.g., miRNA / microRNA) from animal cells, tissue samples or formalin-fixed, paraffin-embedded tissue. It can be used to purify total RNA or to prepare samples enriched for small RNA < 100 nucleotides. The quality of the preparation is suitable for cloning, Northern Blotting, miRNA Array hybridization and for the relative quantification of miRNA with RT-PCR (e.g., on the LightCycler® 480 System).

## Time required
Total time required is approx. 30 min (without Proteinase K incubation and deparaffinization in the preparation of paraffin-embedded tissue samples).

## Results
- **Yield**: Variable, depending on sample type (See the table under part IV of “How to use the kit” in this article).
- **Purity**: Purified RNA is free of DNA, protein, salts and other cellular components.

## Benefits
- **Eliminate the need for hazardous organic solvents.** Isolate high-quality RNAs without using toxic phenol/chloroform.
- **Obtain high yields with a simple, efficient protocol.** Recover high yields of purified total RNA and miRNA through a straightforward protocol.
- **Choose one flexible kit for all your miRNA purifications.** Use the same versatile kit to purify small RNAs from a variety of sample types, including both fresh and FFPE tissues.
How to use the kit

I. Flow diagram

1-Column protocol for the isolation of total RNA containing small RNA

150 μl lysate supernatant
- Mix well and apply mixture (max. 700 μl at a time) to a High Pure Filter Tube assembly, centrifuge at 13,000 × g for 30 - 60 s (repeat if lysate volume is more than 700 μl).

Discard flowthrough
- Centrifuge at 13,000 × g for 30 s

Discard flowthrough
- Add 500 μl Wash Buffer

Discard flowthrough
- Add 300 μl Wash Buffer

Discard flowthrough
- Add 300 μl Wash Buffer

Discard collection tube
- Centrifuge at 13,000 × g for 1 min

Place the Filter Tube in a fresh 1.5 ml microcentrifuge tube
- Incubate for 1 min at +15 to +25°C.
- Centrifuge at 13,000 × g for 1 min

Add 50 - 100 μl Elution Buffer on the center of the filter

Purified total RNA
2-Column protocol for the isolation of small RNA <100 nucleotides

1. **150 μl lysate supernatant**
   - Add 312 μl Binding Buffer
   - Mix well and apply mixture (max. 700 μl at a time) to a High Pure Filter Tube assembly, centrifuge at 13,000 × g for 30 – 60 s (repeat if lysate volume is more than 700 μl).

2. **Collect flowthrough**
   - Add 200 μl Binding Enhancer
   - Mix well and apply mixture to a new High Pure Filter Tube assembly, centrifuge at 13,000 × g for 30 s.

3. **Centrifuge at 13,000 × g for 30 s**
   - Add 500 μl Wash Buffer
   - Add 300 μl Wash Buffer

4. **Discard flowthrough**
   - Centrifuge at 13,000 × g for 30 s
   - Centrifuge at 13,000 × g for 1 min

5. **Discard collection tube**
   - Place the Filter Tube in a fresh 1.5 ml microcentrifuge tube
   - Add 50 - 100 μl Elution Buffer on the center of the filter
   - Incubate for 1 min at +15 to +25°C.
   - Centrifuge at 13,000 × g for 1 min

6. **Purified small RNA**
II. Kit contents

- Paraffin Tissue Lysis Buffer, 20 ml (for FFPE sections only)
- Proteinase K, Lyo 100 mg (for FFPE sections only)
- Binding Buffer, 80 ml, contains guanidine-thiocyanate
- Binding Enhancer, 20 ml
- Wash Buffer 2, 10 ml; add 40 ml absolute ethanol to each vial of Wash Buffer
- Elution Buffer, 30 ml, PCR grade water
- High Pure Filter Tubes, 2 bags with 50 columns for processing up to 700 μl sample volume
- Collection Tubes, 2 bags with 50 polypropylene tubes (2 ml)

\(^{1}\)FFPE formalin-fixed, paraffin-embedded

⚠️ All solutions are clear. If any solution contains a precipitate, warm the solution prior to use at room temperature or in a +37°C water bath to dissolve the precipitate.

⚠️ Do not use vessels or pipettes containing polystyrene (PS) when working with the Binding Enhancer (vial 4)

III. Additional materials needed

- Absolute ethanol
- 10 % SDS solution (for FFPE sections only)
- Hemo-De or Xylene (for FFPE sections only)
- Standard tabletop microcentrifuge capable of 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml/2.0 ml, sterile
- Mortar and Pestle, MagNA Lyser Instrument or Rotor-Stator Homogenization device (e.g., Ultra Turrax)

IV. Typical RNA yield from different sample types

<table>
<thead>
<tr>
<th>Type of tissue</th>
<th>Yield of total RNA (1 column protocol) [μg/mg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse liver</td>
<td>1.5 — 9</td>
</tr>
<tr>
<td>Mouse kidney</td>
<td>0.5 — 7</td>
</tr>
<tr>
<td>Rat liver</td>
<td>1.5 — 8</td>
</tr>
<tr>
<td>Rat brain</td>
<td>0.5 — 2</td>
</tr>
<tr>
<td>Rat muscle</td>
<td>0.5 — 3.5</td>
</tr>
<tr>
<td>Rat heart</td>
<td>0.5 — 2.5</td>
</tr>
<tr>
<td>K562 cells</td>
<td>15 — 30 μg per 10⁶ cells</td>
</tr>
</tbody>
</table>
V. Protocols for preparing total RNA including miRNA or enriched miRNA

Va. Protocols for the isolation of microRNA from tissue

One-Column Protocol (total RNA)

1. To prepare the sample binding buffer mixture
   - Take 150 μl of cell lysate
   - For optimum results do not add lysate from more than 10 mg of animal tissue, 50 mg of plant tissue, or 10⁶ animal or plant cells to the column at this step.
   - Add 312 μl Binding Buffer, vortex briefly, then add 200 μl Binding Enhancer.
   - Vortex 3 × 5 s

2. To transfer the sample to a High Pure Tube
   - Combine the High Pure Filter Tube with a collection tube and pipette the whole mixture from step 1 into the upper reservoir.

3. Centrifuge for 30 s at 13,000 × g, discard the flowthrough.

   *Step 2 – 3 can be repeated in order to load the column with additional sample material (do not overload the column and avoid clumping on the filter fleece).*

4. Add 500 μl Wash Buffer working solution.
   - Centrifuge for 30 s at 13,000 × g, discard the flowthrough.

5. After first wash:
   - Add 300 μl Wash Buffer working solution.
   - Centrifuge for 30 s at 13,000 × g, discard the flowthrough.

6. To dry the glass fiber fleece:
   - Centrifuge at 13,000 × g for 1 min.

7. To elute the RNA:
   - Place the High Pure Filter Tube in a fresh 1.5 ml microcentrifuge tube,
   - Add 100 μl Elution Buffer and incubate for 1 min at +15 to +25°C.
   - To increase RNA concentration of your sample, elution with 50 μl Elution Buffer is possible. An elution step with 100 μl Elution Buffer will increase the total yield by approx. 10 %.
   - Centrifuge for 1 min at approx. 13,000 × g.

The microcentrifuge tube now contains the eluted total RNA. Either use 0.5 – 5 μl of the eluted RNA directly in RT-PCR or store the eluted RNA at −80°C for later analysis.

Glass fibers in the eluate may interfere with optical density measurement. Before determining the RNA concentration photometrically, centrifuge and transfer supernatant to a fresh 1.5 ml reaction tube.
Two-Column Protocol (purified, enriched small RNA)

1. To prepare the sample, binding buffer mixture
   - Take 150 μl of cell lysate
     - For optimum results do not add lysate from more than 10 mg of animal tissue, 50 mg of plant tissue, or 10^6 animal or plant cells to the column at this step.
   - Add 312 μl Binding Buffer, vortex briefly, then add 200 μl Binding Enhancer.
   - Vortex 3 × 5 s

2. To transfer the sample to a High Pure Tube
   - Combine the High Pure Filter Tube with a collection tube and pipette the whole mixture from step 1 into the upper reservoir.
   - Centrifuge for 30 s at 13,000 × g, discard the flowthrough.
     - To minimize pipetting steps a 2 ml microcentrifuge tube can be used instead of the collection tube.
   - Add 200 μl Binding Enhancer.
   - Vortex 3 × 5 s.

3. Combine a new High Pure Filter Tube with a collection tube and pipette the whole mixture from step 3 into the upper reservoir.
   - Centrifuge for 30 s at 13,000 × g, discard the flowthrough.
     - Steps 2 – 4 can be repeated in order to load the column with additional sample material (do not overload the column).

4. Prepare the first wash
   - Add 500 μl Wash Buffer working solution.
   - Centrifuge for 30 s at 13,000 × g, discard the flowthrough.

5. After the first wash:
   - Add 300 μl Wash Buffer working solution.
   - Centrifuge for 30 s at 13,000 × g, discard the flowthrough.

6. To dry the glass fiber fleece:
   - Centrifuge at 13,000 × g for 1 min.

7. To elute the RNA:
   - Place the High Pure Micro Filter Tube in a fresh 1.5 ml microcentrifuge tube, add 100 μl Elution Buffer and incubate for 1 min at +15 to +25°C.
     - To increase RNA concentration of your sample, elution with 50 μl Elution Buffer is possible. An elution step with 100 μl Elution Buffer will increase the total yield by approx. 30 %.
   - Centrifuge for 1 min at 13,000 × g.
     - The microcentrifuge tube now contains the eluted microRNA. Either use 0.5 – 5 μl of the eluted RNA directly in RT-PCR or store the eluted (microRNA) at −80°C for later analysis.
Vb. Protocol for the isolation of microRNA from formalin-fixed, paraffin-embedded tissue

1. To prepare the sample binding buffer mixture
   - Add 100 μl Paraffin Tissue Lysis Buffer, 16 μl 10% SDS and 40 μl Proteinase K working solution to each deparaffinized sample. For a deparaffinization protocol of FFPE slices please review the package insert.
   - Vortex 3 × 5 s.
   - To increase yield, incubate at +55°C for at least 3 h (up to overnight).
   - Add 325 μl Binding Buffer
   - Vortex briefly
   - Add 120 μl Binding Enhancer, vortex 3 × 5 s.

2. To transfer the sample to a High Pure Tube
   - Combine the High Pure Filter Tube with a 2 ml collection tube and pipette the lysate into the upper reservoir.
   - To minimize pipetting steps a 2 ml microcentrifuge tube can be used instead of the collection tube.
   - Centrifuge for 30 s at 13,000 × g in a microcentrifuge and

3. Collect the flowthrough.
   - Add 205 μl Binding Enhancer
   - Vortex 3 × 5 s.
   - Combine the High Pure Filter Tube with a collection tube and pipette the whole mixture from step 6 into the upper reservoir.
   - Centrifuge for 30 s at 13,000 × g, discard the flowthrough.
   - Steps 2 - 3 can be repeated in order to load the column with additional sample material (do not overload the column).

4. Prepare the first wash:
   - Add 500 μl Wash Buffer working solution.
   - Centrifuge for 30 s at 13,000 × g, discard the flowthrough.

5. After the first wash:
   - Add 300 μl Wash Buffer working solution.
   - Centrifuge for 30 s at 13,000 × g, discard the flowthrough.

6. To dry the glass fiber fleece:
   - Centrifuge at 13,000 × g for 1 min, in order to dry the filter fleece completely.

7. To elute the RNA:
   - Place the High Pure Filter Tube in a fresh 1.5 ml microcentrifuge tube.
   - Add 100 μl Elution Buffer and incubate for 1 min at +15 to +25°C.
   - To increase RNA concentration of your sample, elution with 50 μl Elution Buffer is possible.
   - Centrifuge for 1 min at 13,000 × g.
   - The microcentrifuge tube now contains the eluted microRNA. Either use 0.5 – 5 μl of the eluted RNA directly in RT-PCR or store the eluted microRNA at −80°C for later analysis.
Vc. Protocol for the optional treatment of the eluate with DNase

For DNase treatment it is recommended to elute the sample with 100 μl Elution Buffer to minimize loss of sample material.

1. Preparation of DNase Solution:
   - For 70 μl DNase solution mix 49.5 μl PCR grade water, 19.5 μl 10 × DNase Incubation buffer and 1 μl DNase 1 (10 U/μl).
   - DNase treatment on the column is not recommended as miRNA will elute at low salt concentrations.

2. Preparation of the reaction mix:
   - To approx. 100 μl eluate add 56 μl DNase Solution.
   - Vortex 3 × 5 s.
   - Incubate for 30 min at +37°C.

3. For reaction cleanup:
   - Add 325 μl Binding Buffer.
   - Vortex briefly.
   - Add 210 μl Binding Enhancer, vortex 3 × 5 s.

4. Combine the High Pure Filter Tube with a 2 ml collection tube and pipette the DNase treated sample into the upper reservoir.
   - Centrifuge for 30 s at 13,000 × g in a microcentrifuge and discard the flowthrough.

5. Prepare the first wash:
   - Add 500 μl Wash Buffer working solution.
   - Centrifuge for 30 s at 13,000 × g, discard the flowthrough.

6. After the first wash:
   - Add 300 μl Wash Buffer working solution.
   - Centrifuge for 30 s at 13,000 × g, discard the flowthrough.

7. To dry the glass fiber fleece:
   - Centrifuge at 13,000 × g for 1 min, in order to dry the filter fleece completely.

8. To elute the RNA:
   - Place the High Pure filter tube in a fresh 1.5 ml microcentrifuge tube, add 100 μl Elution Buffer and incubate for 1 min at +15 to +25°C.
   - To increase RNA concentration of your sample, elution with 50 μl Elution Buffer is possible. An elution step with 100 μl Elution Buffer will increase the total yield by approx. 10%.
   - Centrifuge for 1 min at 13,000 × g.
   - The microcentrifuge tube now contains the eluted microRNA. Either use 0.5 – 5 μl of the eluted RNA directly in RT-PCR or store the eluted microRNA at –80°C for later analysis.

Glass fibers in the eluate may interfere with optical density measurement. Before determining the RNA concentration photometrically, centrifuge and transfer supernatant to a fresh 1.5 ml reaction tube.

VI. Troubleshooting the High Pure Protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocol, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual.
Typical results with the kit

Experiment 1

Figure 23: The integrity and size distribution of total RNA and small RNA (2-column protocol) purified with the High Pure miRNA Isolation Kit have been checked electrophoretically on a denaturing gel (15% acrylamide/TBE/urea). A 1 μg sample of miRNA 145 was spiked into a liver tissue lysate before purification. Nucleic acids were visualized by ethidium bromide staining. Alternatively, electropherograms were recorded on an Agilent Bioanalyzer (Fig 25).

Eluate volume: 100 μl; 10 μl sample per lane
Eluate 1: 1-Column protocol; total RNA
Eluate 2: 2-Column protocol; high molecular weight RNA eluted from the first filter tube (control of purification)
Eluate 3: 2-Column protocol; purified small RNA (miRNA enriched)

Result: Purified small RNA (2-column protocol) is free of DNA, nucleases and all cellular and sample contaminants that interfere with RT-PCR. The absence of contaminating DNA is examined by PCR without a preceding RT-reaction; no amplification product is obtained (data not shown).
High Pure miRNA Isolation Kit

Typical results with the kit

1-Column protocol; total RNA from 5 mg liver (mouse) stabilized in RNAlater®

2-Column protocol; high molecular weight RNA eluted from the first filter tube (control of purification) from 5 mg liver (mouse) stabilized in RNAlater®

2-Column protocol; purified small RNA (miRNA enriched) from 5 mg liver (mouse) stabilized in RNAlater®

Figure 24: Electropherograms recorded on an Agilent Bioanalyzer

Eluate 1: 1-Column protocol; total RNA
Eluate 2: 2-Column protocol; high molecular weight RNA eluted from the first filter tube (control of purification)
Eluate 3: 2-Column protocol; purified small RNA (miRNA enriched)
Experiment 2

Figure 25: Isolation of total RNA from 8 different mice organs. Half of the organs were fresh-frozen, and the other half formalin-fixed and paraffin-embedded. Fresh frozen samples were extracted with a phenol / chloroform reagent for comparison. Equal amounts (10 μm sections) of FFPE tissue were used for comparison of the High Pure miRNA Isolation Kit with 2 kits from alternative suppliers. All kits were applied according to manufacturer’s recommendation. Isolated total RNA from each preparation was quantified on a Nanodrop instrument.

Results: The High Pure miRNA Isolation Kit shows an overall superior yield compared with kits from 2 alternative supplier and no significant loss compared with a phenol / chloroform based isolation method (data kindly provided by Exiqon A/S).

Reference


Santosh K et al. (2010) Cancer Res., 70, 36 – 45

High Pure Viral RNA Kit

for isolation of RT-PCR templates from up to 100 samples
Cat. No. 11 858 882 001

Principle
Viruses, when lysed by detergent, release viral RNA. Then, in the presence of a chaotropic salt (guanidine HCl), viral RNA binds selectively to glass fiber fleece in a special centrifuge tube. The RNA remains bound while a series of rapid “wash-and-spin” steps remove contaminating cellular components. Finally, low salt elution removes the NA from the glass fiber fleece. The process does not require RNA precipitation, organic solvent extractions, or extensive handling of the RNA.

Starting material
Research samples (200 – 600 µl) may be:
- Serum
- Plasma
- Tears
- Cell culture supernatant
- Urine
- Breast milk

Application
- Preparation of intact viral genomic RNA, which may be used directly as templates for RT-PCR.

RNA preparations obtained are suitable for RT-PCR; they are not tested for other applications

Time required
- Total time: approx. 10 min
- Hands-on time: <10 min

Results
- Yield: 50 µl eluate is enough for 8 – 14 RT-PCRs.
- Purity: Purified RNA is ready to use in RT-PCR.

Benefits
- Saves time, because the kit can prepare multiple RT-PCR templates in just minutes.
- Accommodates a wide variety of samples, because the same kit can purify viral RNA from several bodily fluids.
- Minimizes RNA loss, because the kit removes contaminants without time-consuming precipitation or solvent extraction.
- Increases lab safety, because the kit minimizes the handling of potentially hazardous samples and does not use hazardous organic solvents.
How to use the kit

I. Flow diagram

II. Kit contents

- Binding Buffer containing guanidine HCl and Triton X-100 (2 x 25 ml)
- Carrier RNA, Poly(A), lyophilized (2 mg)
- Inhibitor Removal Buffer (33 ml)
  Add 20 ml absolute ethanol to buffer before use.
- Wash Buffer (2 x 10 ml)
  Add 40 ml absolute ethanol to each Wash Buffer before use.
- Elution Buffer (30 ml)
- High Pure Filter Tubes (100 tubes)
- Collection Tubes, 2 ml (400 tubes)
III. Additional materials needed

- Absolute ethanol
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, nuclease-free

IV. Protocol for preparing RNA from 200 μl samples

See the package insert supplied with the kit for instructions on processing 200 – 600 μl samples.

1. To a nuclease-free 1.5 ml microcentrifuge tube:
   - Add 200 μl serum or plasma.
   - Add 400 μl working solution [Carrier RNA-supplemented Binding Buffer] and mix well.
   - The RNA yield can be increased two-fold with an optional incubation step, thus resulting in higher sensitivity. After adding the Binding Buffer to the sample, simply incubate the mixture at +15 to +25°C for 10 min. This incubation step can be omitted when time to result is critical.

2. To transfer the sample to a High Pure Tube:
   - Insert one High Pure Filter Tube into one Collection Tube.
   - Pipette entire sample into upper buffer reservoir of the Filter Tube.

3. Insert the entire High Pure Tube assembly into a standard tabletop microcentrifuge, then centrifuge the tube assembly for 15 s at approx. 8000 x g.

4. After centrifugation:
   - Remove the Filter Tube from the Collection Tube and discard the Collection Tube.
   - Insert the Filter Tube into a new Collection Tube.

5. After reinserting the Filter Tube:
   - Add 500 μl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube assembly and centrifuge 1 min at 8000 x g.
   - Discard flowthrough and combine Filter Tube with a new Collection Tube.

6. After removal of inhibitors:
   - Add 450 μl Wash Buffer to the upper reservoir of the Filter Tube.
   - Repeat the centrifugation (as in step 3).

7. After the first wash and centrifugation:
   - Repeat the discard step (Step 4).
   - Repeat the wash step (Step 6).
   - Leave the Filter Tube-Collection Tube assembly in the centrifuge and spin it for 10 s at maximum speed (approx. 13,000 x g) to remove residual Wash Buffer.

8. Discard the Collection Tube and insert the Filter Tube in a clean, nuclease-free 1.5 ml microcentrifuge tube.

9. To elute the viral RNA:
   - Add 50 μl of Elution Buffer to the upper reservoir of the Filter Tube.
   - Centrifuge the tube assembly for 1 min at 8000 x g.
The microcentrifuge tube now contains the eluted viral RNA. You may:

- EITHER use an aliquot of the eluted RNA directly in RT-PCR
- Use 3.5 – 6 μl of the eluate for the reverse transcriptase reaction.
- OR store the eluted RNA for later analysis at –80°C.

V. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocol, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure Viral RNA Kit, see page 120.

Typical result with the kit

The kit was used to prepare genomic RNA from viruses [for example, hepatitis C virus (HCV), hepatitis G virus (HGV), and human immunodeficiency virus (HIV)] for research applications. Each preparation was used as a template in RT-PCR. All these templates produced highly specific PCR products in good yield.

Figure 26: RT-PCR analysis of MS2 RNA isolated with the High Pure Viral RNA Kit. Serial dilutions of purified MS2 RNA were applied to the Filter Tubes, washed and eluted following the kit protocol. 3.5 μl of the 50 μl eluate were analyzed by two step RT-PCR using primers that resulted in a fragment of 961 bp. The indicated numbers of molecules per PCR correspond to assumed quantitative recovery.

Lane 1: $3.5 \times 10^7$ molecules/PCR;
Lane 2: $3.5 \times 10^5$ molecules/PCR;
Lane 3: $3.5 \times 10^3$ molecules/PCR
Lane 4: $3.5 \times 10^2$ molecules/PCR
Lane 5: 35 molecules/PCR
Lane M: DNA Molecular Weight Marker III

Result: Even with theoretically 35 molecules recovered, a detectable signal in agarose gel electrophoresis is obtained.
References

High Pure Viral Nucleic Acid Kit

for isolation of PCR or RT-PCR templates from up to 100 samples
Cat. No. 11 858 874 001

**Principle**
Viruses, when lysed by detergent and Proteinase K, release total viral nucleic acids (NA). Then, in the presence of a chaotropic salt (guanidine HCl), viral NA binds selectively to glass fiber fleece in a special centrifuge tube. The NA remains bound while a series of rapid “wash-and-spin” steps remove contaminating cellular components. Finally, low salt elution removes the NA from the glass fiber fleece. The process does not require NA precipitation, organic solvent extractions, or extensive handling of the NA.

**Starting material**
Research samples may be 200 – 600 μl of:
- Serum
- Plasma
- Whole blood
- Cell culture supernatant

**Application**
- Preparation of highly purified viral nucleic acids (RNA or DNA), which may be used directly as templates for standard or long-template PCR or RT-PCR

* Nucleic acid preparations obtained are suitable for PCR or RT-PCR; they are not tested for other applications.

**Time required**
- Total time: approx. 20 min
- Hands-on time: <10 min

**Results**
- Yield: 50 μl eluate is enough for 8 – 14 RT-PCRs or 2 – 5 PCRs.
- Purity: Purified NA is free of intact virus, nucleases, and all cellular components that interfere with RT-PCR or PCR.

**Benefits**
- **Saves time**, because the kit can prepare multiple PCR/RT-PCR templates in just minutes.
- Accommodates a wide variety of samples, because the same kit can purify nucleic acids from either DNA or RNA viruses.
- Minimizes loss of nucleic acids, because the kit removes contaminants without precipitation, solvent extraction, or other handling steps that can lead to lost or degraded nucleic acids.
- Increases lab safety, because the kit minimizes the handling of potentially hazardous samples and does not use hazardous organic solvents.
How to use the kit

I. Flow diagram

- **200 μl serum, plasma or whole blood**
  - Mix immediately and incubate for 10 min at +72°C then mix samples with 100 μl Binding Buffer
  - Combine the High Pure Filter Tube and the Collection Tube and pipette the sample in the upper reservoir
  - Centrifuge at 8000 x g for 1 min
  - Discard flowthrough and Collection Tube
  - Centrifuge at 8000 x g for 1 min
  - Add 500 μl Inhibitor Removal Buffer
  - Discard flowthrough and Collection Tube
  - Add 450 μl Wash Buffer
  - Discard flowthrough and Collection Tube
  - Add 450 μl Wash Buffer
  - Discard flowthrough and Collection Tube
  - Centrifuge at 8000 x g for 1 min
  - Centrifuge at max. speed (13,000 x g) for 10 seconds
  - Add 50 μl Elution Buffer
  - Discard flowthrough and Collection Tube
  - Centrifuge at 8000 x g for 1 min

- **Purified Viral Nucleic Acids**

Add 200 μl Binding Buffer supplemented with Poly (A) and 50 μl Proteinase K
II. Kit contents

- Binding Buffer containing guanidine HCl and Triton X-100 (2 x 25 ml)
- Carrier RNA, Poly(A), lyophilized (2 ml)

**Poly(A) Carrier RNA, if dissolved in Binding Buffer at high concentration, will precipitate when stored. Therefore, dissolve the Poly(A) Carrier RNA in Elution Buffer:**

- Add 0.5 ml Elution Buffer to the Carrier RNA vial. Stopper and invert the vial until all the Carrier RNA (including any that might stick to the rubber stopper) is completely dissolved. Pipette 50 μl aliquots of the reconstituted Carrier RNA into separate nuclease-free microcentrifuge tubes and store at −15 to −25°C. Before each experiment, thaw one tube of reconstituted Carrier RNA and add the contents to 2.5 ml Binding Buffer. This working solution may be stored at +15 to +25°C, but must be used within a few days.

- Proteinase K, lyophilized (100 mg)

**Dissolve proteinase K in 5 ml Elution Buffer and store aliquots at −15 to −25°C.**

- Inhibitor Removal Buffer (33 ml)

**Note: Add 20 ml absolute ethanol to buffer before use.**

- Wash Buffer (2 x 10 ml)

**Note: Add 40 ml absolute ethanol to each Wash Buffer before use.**

- Elution Buffer (30 ml)

- High Pure Filter Tubes (100 tubes)

- Collection Tubes, 2 ml (400 tubes)

III. Additional materials needed

- Absolute ethanol
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, nuclease-free

IV. Protocol for preparing nucleic acids from 200 μl samples of serum, plasma, or whole blood (research samples)

See the package insert supplied with the kit for detailed instructions on adapting this protocol to process 200 – 600 μl samples.

1. To a nuclease-free 1.5 ml microcentrifuge tube:
   - Add 200 μl serum, plasma, or whole blood.
   - Add 200 μl working solution [Carrier RNA-supplemented Binding Buffer].
   - Add 50 μl reconstituted Proteinase K solution and mix the contents of the tube immediately.
   - Incubate for 10 min at +72°C.

2. After the incubation, mix the samples with 100 μl Binding Buffer.
To transfer the sample to a High Pure Tube:
- Insert one High Pure Filter Tube into one Collection Tube.
- Pipette entire sample into upper buffer reservoir of the Filter Tube.

Insert the entire High Pure Tube assembly into a standard tabletop microcentrifuge, then centrifuge the tube assembly for 1 min at 8000 x g.

After centrifugation:
- Remove the Filter Tube from the Collection Tube and discard the Collection Tube.
- Insert the Filter Tube into a new Collection Tube.

After reinserting the Filter Tube:
- Add 500 μl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube assembly. Centrifuge for 1 min at 8000 x g (as in Step 4).
- Discard Collection Tube and flowthrough. Reinsert Filter Tube into a new Collection Tube.

After removal of inhibitors:
- Add 450 μl Wash Buffer to the upper reservoir of the Filter Tube.
- Repeat the centrifugation (as in Step 4).

After the first wash and centrifugation:
- Repeat the discard step (Step 5).
- Repeat the wash step (Step 6).
- Leave the High Pure tube assembly in the centrifuge and spin it for 10 s at maximum speed (approx. 13,000 x g) to remove residual Wash Buffer.

Discard the Collection Tube and insert the Filter Tube in a clean, nuclease-free 1.5 ml microcentrifuge tube.

To elute the nucleic acid:
- Add 50 μl of Elution Buffer to the upper reservoir of the Filter Tube.
  - **If the starting sample (Step 1) was whole blood, prewarm the Elution Buffer to +70°C before adding it to the Filter Tube.**
- Centrifuge the tube assembly for 1 min at 8000 x g.

The microcentrifuge tube now contains the eluted, stable viral nucleic acids. You may:
- EITHER use an aliquot of the eluted nucleic acids directly in PCR or RT-PCR
  - **If the nucleic acids are viral RNA, use 3.5 – 6 μl of the eluate for the reverse transcriptase reaction. In case of viral DNA, use 10 – 25 μl of the eluate for PCR.**
- OR store the eluted nucleic acids for later analysis at either +2 to +8°C (viral DNA) or −80°C (viral RNA).

V. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocol, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure Viral Nucleic Acid Kit, see page 121.
Typical results with the kit

The kit was used to prepare nucleic acids from both DNA viruses [for example, hepatitis B virus (HBV) and cytomegalovirus (CMV)] and RNA viruses [for example, hepatitis C virus (HCV), hepatitis G virus (HGV), and human immunodeficiency virus (HIV)] for research applications. Each preparation was used as a template in PCR (DNA) or RT-PCR (RNA). All these templates produced highly specific PCR products in good yield.

Figure 27: Sensitivity of viral RNA detection. For research purposes serial dilutions of HGV, HCV, and HIV serum samples were processed according to the kit protocol. 20 μl of 100 μl eluates were analyzed by one step RT-PCR using either Tth DNA Polymerase (in case of HCV and HIV) or a mixture of AMV Reverse Transcriptase and Taq DNA Polymerase (in case of HGV). PCR products were quantified by electrochemiluminescent detection using a biotinylated capture probe and a ruthenium-labeled detection probe.

Result: Assuming quantitative recovery, detection sensitivity is in the range of 20 – 40 viral copies per PCR.

References

High Pure Viral Nucleic Acid Large Volume Kit

for isolation of PCR or RT-PCR templates from up to 40 samples
Cat. No. 05 114 403 001

Principle
As a prerequisite for PCR or RT-PCR, viral nucleic acids must be isolated from serum, plasma, or whole blood. Viral lysis is accomplished by incubating the sample in a special Lysis/Binding Buffer in the presence of Proteinase K. After applying the lysis mixture to a High Pure Extender Assembly, centrifugation is performed in a tabletop centrifuge. During the spin, the lysis mixture passes through the glass-fiber fleece of the High Pure Spin Column, and nucleic acids are bound to the silica fibers. After the first centrifugation, the High Pure Spin Column is removed from the Extender Assembly and further processed in a microcentrifuge using 2 ml Collection Tubes. During this process, nucleic acids are purified from salts, proteins, and other impurities by washing and centrifugation steps. The bound nucleic acids are eluted in PCR grade water and collected after centrifugation.

Starting material
Purification of viral nucleic acids from up to 2.5 ml
- Serum
- Plasma
- Whole blood

Centrifuge samples containing precipitates before purification.

Application
The High Pure Viral Nucleic Acid Large Volume Kit is designed to efficiently purify viral nucleic acids from serum, plasma, or whole blood. Based on the same principle as the High Pure Viral Nucleic Acid Kit, the kit features an innovative spin column assembly to purify larger sample volumes of up to 2.5 ml. When using whole blood as starting material, total nucleic acids are isolated, including viral nucleic acids. For optimal results, the first step of the isolation process should be performed in a tabletop centrifuge with a swinging bucket rotor capable of holding 50 ml tubes. The purified viral nucleic acid is eluted in PCR grade water, and is suitable for direct use in PCR and RT-PCR.

Time required
- Total time: approx. 25 min
- Hands-on time 10 min

Results
- Yield: 50 μl eluate is enough for 8 – 14 RT-PCRs or 2 – 5 PCRs.
- Purity: Purified NA is free of intact virus, nucleases, and small cellular components that interfere with RT-PCR or PCR.

Benefits
- Improve sensitivity. Use sample volumes up to 2.5 ml to obtain high yields of purified nucleic acid in a concentrated 50 μl eluate.
- Obtain high-purity nucleic acids. Reduce carryover risk by using high centrifugal forces in all wash steps.
- Increase convenience and improve time to result. Eliminate complicated sample pre-processing and rapidly recover purified samples using high-speed centrifugation.
How to use the High Pure Extender Assembly

The High Pure Extender Assembly is delivered in single zip-bags. Five High Pure Extender Assemblies are additionally packed in labeled zip-bags. Each High Pure Extender is assembled in a 50 ml polypropylene Tube. The High Pure Extender Assembly is designed for use with table-top centrifuges and swing-bucket rotors with 4,000 x g force applicable.

Figure 29: High Pure Extender Assembly
Remove the High Pure Extender Assembly from the zip-bags prior to use. In order to load the sample onto the Assembly unscrew the cap of the 50 ml polypropylene tube. After sample loading, close the High Pure Extender Assembly with the 50 ml polypropylene tube cap. After the first centrifugation step, the sample has passed through the High Pure Extender Assembly and is collected at the bottom of the 50 ml polypropylene tube. Nucleic acids are bound to the silica fleece at the bottom of the High Pure filter tube. For further processing remove the High Pure Extender Assembly from the 50 ml polypropylene tube. Discard the tube containing the flow-through. Remove the High Pure Filter Tube from the High Pure Extender Assembly (see Figures 31 to 35).

Disassembly of the High Pure Extender Assembly

Figure 30: Unscrew the 50 ml polypropylene tube and remove the High Pure Extender Assembly from the 50 ml polypropylene tube.

Figure 31: Place the High Pure Extender in a new collection tube, which is placed securely in a tube rack on the bench.
High Pure Viral Nucleic Acid Large Volume Kit

How to use the kit

Figure 32: Secure the High Pure Extender Assembly with one hand while grasping the locking clip (side a) of the High Pure Filter Tube cap on the opposite side with the other hand. Remove the first part of the locking clip (side a) by screwing the clip in either direction.

Figure 33: Rotate the High Pure Extender Assembly. Remove the second part of the locking clip (side b) by pulling the locking clip away from the Extender Assembly.

Figure 34: Remove the High Pure Extender from the High Pure Filter Tube by tilting the High Pure Extender away from the High Pure Filter tube toward the side without the cap.

We do not recommend the use of fixed-angle rotors in combination with the High Pure Extender Assembly. However, if you decide to use a centrifuge with fixed-angle rotors, do not centrifuge High Pure Extender Assembly above 3000 × g. The use of fixed-angle rotors results in incomplete flow of the liquid through the Assembly. The remaining sample solution therefore stays within the High Pure Spin Column Filter Tube. Remove this remaining liquid by an additional spin of the High Pure Filter Tube in a bench-top centrifuge before the first washing step. For this additional spin remove the High Pure Spin Column from the High Pure Extender Assembly according to Figures 30 to 35.
High Pure Viral Nucleic Acid Large Volume Kit
How to use the kit

I. Flow diagram

1 ml serum, plasma or whole blood

- Mix immediately and incubate for 15 min at +70°C. Then mix sample with 400 μl Binding Buffer

Transfer the sample to the High Pure Extender Assembly

- Centrifuge for 5 min at 4,000 × g

Remove the Extender Assembly from the polypropylene tube. Remove the High Pure Filter Tube from the Extender Assembly. Discharge everything except the High Pure Filter Tube. Place the Filter Tube in a new Collection Tube

- Centrifuge for 1 min at 8,000 × g

- Add 500 μl Inhibitor Removal Buffer

Discharge the flow through and Collection Tube

- Add 450 μl Washing Buffer

- Add 450 μl Washing Buffer

- Discharge the flow through

- Discharge the flow through

- Centrifuge for 30 sec max. speed (13,000 × g)

- Centrifuge for 1 min at 8,000 × g

- Add 50 μl Elution Buffer

Discharge the flow through and Collection Tube

- Centrifuge for 1 min at 8,000 × g

- Purified nucleic acid
II. Kit contents

All solutions are clear. Do not use them when precipitates have formed. Warm the solutions at +15 to +25°C or in a +37°C water bath until the precipitates have dissolved.

- Binding Buffer 6 × 25 ml, 6 M guanidine-HCl, 10 mM Tris-HCl, 20%, Triton® X-100 (w/v), pH 4.4 (+25°C)
- Poly(A) Lyophilizate, 2 mg poly(A) carrier RNA for binding of RNA
- Dissolve poly(A) carrier RNA in 0.65 ml Elution Buffer.
- Prepare 15 μl aliquots. Store aliquots at –15 to –25°C, stable for 12 months
  - For the preparation of the working solution:
    - Thaw one vial with 15 μl poly(A) carrier RNA and mix thoroughly with 0.5 ml to 2.5 ml Binding Buffer according to Table 2 of the isolation protocol.
  - Always prepare freshly before use! Do not store!
- Proteinase K lyophilizate, 2 × 100 mg for the digestion of proteins
- Dissolve Proteinase K in 5.5 ml Elution Buffer and mix thoroughly. Prepare 130 μl aliquots
- Store aliquots at –15 to –25°C, Stable for 12 months
- Inhibitor Removal Buffer, 33 ml, 5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6, (+25°C) final concentration after addition of ethanol
  - Add 20 ml absolute ethanol
- Wash Buffer 10 ml, 20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C), final concentrations after addition of ethanol
  - Add 40 ml ethanol p.a in each case
- Elution Buffer 30 ml PCR grade water
- High Pure Extender Assembly, 8 bags, 5 pieces each in a single zip pack
- Collection Tubes, 2 bags with 50 polypropylene tubes (2 ml)

III. Additional material needed

- Absolute ethanol
- Standard tabletop centrifuge with swing-bucket rotor capable of 5000 × g centrifugal force for 50 ml polypropylene tubes
- Standard tabletop microcentrifuge capable of 13,000 × g centrifugal force (e.g., Eppendorf 5415C or equivalent)
- Microcentrifuge tubes, 1.5 ml, sterile
IV. Protocol for preparing nucleic acids from 1 ml samples of serum, plasma, or whole blood (research samples)

1. To a nuclease-free 15 ml falcon tube
   - Add 1 ml serum, plasma or whole blood
   - Add 1 ml working solution, freshly prepared, (carrier RNA-supplemented Binding Buffer)
   - Add 250 μl Proteinase K solution, and mix immediately.
   - Incubate for 15 min at +70°C.

2. Add 400 μl Binding Buffer and mix. Transfer the sample to a High Pure Extender Assembly.

3. Pipette entire sample into the upper reservoir of the High Pure Extender Assembly.

4. Insert the entire High Pure Filter Tube Assembly into a standard tabletop centrifuge with a swing-bucket rotor.
   - Then centrifuge for 5 min at 4,000 × g.

5. After centrifugation:
   - Remove the Filter Tube from the High Pure Extender Assembly, discard the flowthrough liquid, and the 50 ml falcon tube.
   - Combine the Filter Tube with a new Collection Tube.

6. After combining the Filter Tube with a new Collection Tube:
   - Add 500 μl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube.
   - Centrifuge for 1 min at 8,000 × g.

7. After centrifugation:
   - Remove the Filter Tube from the Collection Tube.
   - Combine the Filter Tube with a new Collection Tube.
   - Discard the Collection Tube including the flowthrough liquid.

8. After removal of inhibitors:
   - Add 450 μl Wash Buffer to the upper reservoir of the Filter Tube.
   - Centrifuge for 1 min at 8,000 × g and discard the flowthrough.

9. After the first wash and centrifugation:
   - Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid.
   - Combine the Filter Tube with a new Collection Tube.
   - Add 450 μl Wash Buffer to the upper reservoir of the Filter Tube.
   - Centrifuge for 1 min at 8,000 × g and discard the flowthrough.
   - Leave the Filter Tube-Collection Tube Assembly in the centrifuge and spin it for 30 s at maximum speed (approx. 13,000 × g) to remove any residual Wash Buffer.

10. The extra centrifugation time ensures removal of residual Wash Buffer.

11. Discard the Collection Tube and insert the Filter Tube into a nuclease-free, sterile 1.5 ml microcentrifuge tube.

12. To elute the viral nucleic acids:
   - Add 50 μl Elution Buffer to the upper reservoir of the Filter Tube.
   - Incubate for 1 min at room temperature.
   - Centrifuge the tube assembly for 1 min at 8,000 × g.
The microcentrifuge tube now contains the eluted, stable viral nucleic acids. You may:

- Either use the eluted nucleic acids directly in PCR (10 – 20 μl DNA eluate) or RT-PCR (3.5 μl viral RNA)
- or store the eluted viral RNA at -80°C or the viral DNA at +2 to +8°C or at -15 to -25°C for later analysis

For isolation of nucleic acids from whole blood use pre-warmed Elution Buffer (+70°C).

The High Pure Extender Assembly is designed for sample volumes up to 2.5 ml. The sample buffer compositions for different sample volumes are listed in the table below.

<table>
<thead>
<tr>
<th>Sample Volume</th>
<th>0.5 ml</th>
<th>1 ml</th>
<th>2.5 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding Buffer</td>
<td>0.5 ml</td>
<td>1 ml</td>
<td>2.5 ml</td>
</tr>
<tr>
<td>Poly A</td>
<td>15 μl</td>
<td>15 μl</td>
<td>15 μl</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>125 μl</td>
<td>250 μl</td>
<td>250 μl</td>
</tr>
<tr>
<td>Binding Buffer (protocol step 2)</td>
<td>0.2 ml</td>
<td>0.4 ml</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

Table 2: Sample buffer compositions for different sample volumes loaded onto the High Pure Extender Assembly.

V. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocol, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure Viral Nucleic Acid Kit, see page 121.
Typical results with the kit

Experiment 1

Figure 35: Generate high-quality template with excellent linearity in qRT-PCR. Citrated plasma research samples were spiked with a dilution series of hepatitis A virus (HAV) in the range of $10^2$ to $10^6$ copies per ml of plasma. Nucleic acids were isolated from 1 ml of spiked plasma using the High Pure Viral Nucleic Acid Isolation Large Volume Kit as described in the kit protocol. Five microliters of eluate from each isolation was used as template in qRT-PCR analysis with the LightCycler® Carousel-Based System.

Result: The template purified using the High Pure Viral Nucleic Acid Isolation Large Volume Kit generated a highly specific PCR product that demonstrates excellent performance and linearity in qRT-PCR.

Experiment 2

Figure 36: Purify nucleic acids from a broad range of sample sizes. Various amounts (200 μl, 1 ml, 25 ml, and 5 ml) of serum research samples were spiked with a dilution series ($1 \times 10^6$ to $1 \times 10^4$ copies/ml) of hepatitis A virus (HAV) particles. Nucleic acids were isolated from each sample using the High Pure Viral Nucleic Acid Large Volume Kit according to the kit protocol. Five microliters of each sample eluate was analyzed by LightCycler® System qRT-PCR.

Result: The data shows the sensitivity and linearity of the purified nucleic acids in qRT-PCR analysis, and consistent performance independent of sample volume. The experiment also demonstrates that the High Pure Viral Nucleic Acid Large Volume Kit can accommodate serum sample volumes as large as 5 ml, yielding highly pure, concentrated nucleic acids.

Reference

Please find relevant references on page 104 in the references section of the High Pure Viral Nucleic Acid Kit.
# Troubleshooting Procedures

## I. Factors that may affect all High Pure kits

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low nucleic acid yield or purity</td>
<td>Kit stored in less than optimal conditions</td>
<td>Store all High Pure kits at +15 to +25°C as soon as they arrive.</td>
</tr>
<tr>
<td></td>
<td>Buffers or other reagents exposed to conditions that lessened their effectiveness</td>
<td>Store all buffers at +15 to +25°C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If the temperature in your laboratory is lowered overnight, warm all buffers at +37°C for 10 to 15 min.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After any lyophilized reagent is reconstituted, aliquot it and store the aliquots at either +2 to +8°C or −15 to −25°C (as directed in kit package insert).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Close all wash buffer bottles tightly to prevent ethanol evaporation during storage.</td>
</tr>
<tr>
<td>Ethanol not added to Wash Buffer</td>
<td>Add absolute ethanol to all Wash Buffers before using (as indicated in kit package insert).</td>
<td>Add absolute ethanol to all Wash Buffers before using (as indicated in kit package insert).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After adding ethanol, mix the Wash Buffer well and store at +15 to +25°C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Always mark the Wash Buffer vial to indicate whether ethanol has been added or not.</td>
</tr>
<tr>
<td>Reagents and sample not completely mixed</td>
<td>Always mix the sample tube well after addition of each reagent.</td>
<td>Always mix the sample tube well after addition of each reagent.</td>
</tr>
<tr>
<td>Poor elution of nucleic acids with water</td>
<td>Water has the wrong pH</td>
<td>If you use PCR grade water to elute nucleic acids from Filter Tube, be sure the water has the same pH as the Elution Buffer supplied in the kit.</td>
</tr>
<tr>
<td></td>
<td>Used a buffer from a different High Pure Kit.</td>
<td>The Elution Buffer from the different High Pure Kits have different pHs depending on the nucleic acid being purified. Swapping buffers may cause low yields.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Recovery can be improved by incubating the column filter with elution buffer for 1 to 5 min after addition of elution buffer.</em></td>
</tr>
<tr>
<td>Incomplete or no restriction enzyme cleavage of product</td>
<td>Glass fibers, which coeluted with the nucleic acid, inhibit enzymes</td>
<td>1. Remove High Pure Filter Tube from tube containing eluted sample and spin sample tube for 1 min at maximum speed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Transfer supernatant to a new tube without disturbing the glass fibers at the bottom of the original tube.</td>
</tr>
</tbody>
</table>
I. Factors that may affect all High Pure kits, continued

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance (A_{260}) reading of product too high</td>
<td>Glass fibers, which coeluted with the nucleic acid, scatter light</td>
<td>▶ See suggestions under “Incomplete or no restriction enzyme cleavage of product” above.</td>
</tr>
</tbody>
</table>
| Samples “pop” out of wells in agarose gel | Eluate contains ethanol (from the Wash Buffer) | ▶ 1. After the last wash step, do not let the flowthrough (used Wash Buffer) touch the bottom of the High Pure Filter Tube.  
▶ 2. Empty Collection Tube, reinsert Filter Tube in emptied Collection Tube, and recentrifuge for 30 s. |

II. Factors that may affect the High Pure PCR Template Preparation Kit

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
</table>
| Low yield from any starting material | Proteinase K not completely solubilized | To solubilize the lyophilized Proteinase K completely:  
▶ 1. Pipette 4.5 ml of PCR grade water into the glass vial containing lyophilized Proteinase K.  
▶ 2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.  
▶ 3. Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at –15 to –25°C.  
▶ Reconstituted Proteinase K is stable for 12 months when stored properly. |
| Incomplete lysis | | ▶ After adding Proteinase K to sample, mix immediately.  
▶ Always mix lysate well with isopropanol before adding it to the High Pure Filter Tube. |
| Low yield from tissue | Incomplete Proteinase K digestion | ▶ Cut tissue into small pieces before digestion and lysis.  
▶ Increase incubation time with Proteinase K (Step 1 of Protocol Vb) in either of two ways:  
▶ Incubate tissue with Proteinase K overnight.  
▶ Incubate with Proteinase K for 3 – 4 h, then add a fresh aliquot of Proteinase K (30 μl) and incubate another 1 – 2 h.  
▶ To accommodate increased volume (sample + enzyme), use 230 μl Binding Buffer instead of 200 μl in Step 2 of Protocol Vb. |
| Low yield from bacteria or yeast | Cells not lysed efficiently with lysozyme (bacteria) or lyticase (yeast) | ▶ Make sure your cells can be lysed by lysozyme or lyticase. The kit package insert lists some recommended strains (which are known to be lysed by the enzymes).  
▶ Use alternative lysis procedures, such as mechanical grinding, vortexing with glass beads, boiling, or repeated freeze-thaw. |
# II. Factors that may affect the High Pure PCR Template Preparation Kit, continued

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
</table>
| Degraded DNA from tissue samples | Nuclease activity in unlysed tissue | ▶ Tissue should be frozen (−15 to −25°C) from the time of harvest until the lysis procedure starts.  
▶ Use only small pieces of tissue (20 – 40 mg) in the procedure, or homogenize the tissue sample. |
| Eluate from blood samples is still slightly colored | Incomplete wash | ▶ Wash Filter Tube until flowthrough is colorless.  
▶ Repeat purification protocol by mixing 200 μl eluate with 200 μl Binding Buffer, then 100 μl isopropanol. Follow Protocol Va (page 23), starting with the application of the sample to the High Pure Filter Tube (Step 3).  
⚠️ Omit Proteinase K digestion and +72°C incubation. |

# III. Factors that may affect the High Pure PCR Cleanup Micro Kit

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
</table>
| Low recovery of nucleic acids in eluate | Non-optimal reagent has been used for elution | ▶ Do not use water to elute nucleic acids from Filter Tube.  
▶ Alkaline pH is required for optimal elution.  
▶ Use the Elution Buffer in the kit. |
| Concentration of DNA in the eluate is too low | Low concentrations of amplified DNA were added to the High Pure Filter Tube (in Step 1) | Verify PCR result by agarose gel electrophoresis before starting purification procedure.  
⚠️ Do not use less than 10 μl Elution Buffer |
| No PCR product in final eluate | No PCR product in starting material | Verify PCR result by agarose gel electrophoresis before starting purification procedure. |
| Short read length in sequencing reactions | Copurification of DNA binding proteins | Use the Expand High Fidelity PCR System for template synthesis.  
Use Binding buffer without Binding Enhancer for purification. |
| | Copurification of primer and primer-dimer | |
## IV. Factors that may affect the High Pure PCR Product Purification Kit

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low yield</td>
<td>Reagents exposed to conditions that lessened their effectiveness</td>
<td>Store all buffers at +15 to +25°C.</td>
</tr>
<tr>
<td></td>
<td>Reagent and sample not completely mixed</td>
<td>Always mix contents of sample tube well after each reagent addition.</td>
</tr>
<tr>
<td></td>
<td>Too little Binding Buffer used</td>
<td>Make sure the ratio of PCR product to Binding Buffer is 1:5. Oil overlay, wax, and gel loading dye do not interfere with the purification procedure.</td>
</tr>
<tr>
<td></td>
<td>Incomplete elution</td>
<td>Elute product with two volumes of Elution Buffer (50 μl each), centrifuging after addition of each volume.</td>
</tr>
</tbody>
</table>

### Concentration of DNA in eluate too low
- Too much Elution Buffer
  - Decrease volume of Elution Buffer.
  - Do not use <50 μl.

### No PCR product in final eluate
- No PCR Product in starting material
  - Check PCR result by agarose gel electrophoresis before starting purification procedure.

## V. Factors that may affect the High Pure Plasmid Isolation Kit

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low plasmid yield</td>
<td>Too few cells in starting material</td>
<td>Grow <em>E. coli</em> to an absorbance (A_{600}) of 1.0 – 1.9 before harvest.</td>
</tr>
<tr>
<td></td>
<td>Incomplete cell lysis</td>
<td>Be sure the <em>E. coli</em> pellet is completely resuspended in Suspension Buffer.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Make sure the lysate is clear and viscous after the lysis step (incubation with Lysis Buffer).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Make sure a cloudy white precipitate forms when Binding Buffer is added to lysate. The precipitate should pellet completely during centrifugation.</td>
</tr>
<tr>
<td></td>
<td>Lysate did not bind completely to High Pure Filter Tube</td>
<td>Pre-equilibrate the glass fleece in the Filter Tube by adding 200 μl Binding Buffer to the Filter Tube before applying sample.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Do not centrifuge the Filter Tube after this step. Instead, apply the sample and centrifuge as directed in Step 6 of the protocol.</td>
</tr>
<tr>
<td>Plasmid is degraded or no plasmid obtained</td>
<td>High levels of nuclease activity</td>
<td>Use optional Wash Buffer I (Step 7 of protocol) to eliminate nuclease activity in <em>E. coli</em> strains with high levels of nuclease (for example, HB 101).</td>
</tr>
</tbody>
</table>
### V. Factors that may affect the High Pure Plasmid Isolation Kit, continued

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA present in final product</td>
<td>RNase not completely dissolved</td>
<td>To solubilize the lyophilized RNase completely:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>▶ 1. Pipette 1 ml of Suspension Buffer into the glass vial containing lyophilized RNase.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>▶ 2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>▶ 3. Transfer all the reconstituted RNase back into the Suspension Buffer vial and mix thoroughly.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>▶ 4. Mark the reconstituted mixture (enzyme + buffer) with the date of reconstitution, and store at +2 to +8°C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Reconstituted mixture is stable for 6 months when stored properly.</em></td>
</tr>
<tr>
<td>Too many cells in starting material</td>
<td></td>
<td>Do not use more than 4 ml of an overnight <em>E. coli</em> culture as starting material.</td>
</tr>
<tr>
<td>Genomic DNA present in final product</td>
<td>Genomic DNA sheared during lysis step</td>
<td>At Step 3 of the protocol, mix the Lysis Buffer and sample by gentle inversion of the tube.</td>
</tr>
<tr>
<td>Additional band running slightly faster than supercoiled plasmid is seen on gels</td>
<td>Denatured plasmid in final product</td>
<td>Reduce the incubation time during Step 3 (lysis step) of the protocol.</td>
</tr>
</tbody>
</table>

### VI. Factors that may affect the High Pure RNA Isolation Kit

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>No RNA</td>
<td>High levels of RNase activity</td>
<td>▶ Be careful to create an RNase-free working environment.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>▶ Process starting material immediately or store it at −80°C until it can be processed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>▶ Use eluted RNA directly in downstream procedures or store it immediately at −80°C.</td>
</tr>
<tr>
<td>Cultured cells not completely resuspended</td>
<td></td>
<td>Resuspend cell pellet completely in PBS before starting procedure.</td>
</tr>
</tbody>
</table>
VI.  Factors that may affect the High Pure RNA Isolation Kit, continued

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA present in final product</td>
<td>DNase not completely dissolved</td>
<td>To solubilize the lyophilized DNase completely:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1. Pipette 0.55 ml of Elution Buffer into the glass vial containing lyophilized DNase.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at –15 to –25°C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reconstituted DNase is stable for 12 months when stored properly. To create a working DNase solution for 10 samples, mix 100 μl reconstituted DNase with 900 μl DNase Incubation Buffer.</td>
</tr>
<tr>
<td>DNA not evenly distributed in High Pure Filter Tube</td>
<td>When pipetting, distribute the working DNase solution evenly over the glass fleece in the Filter Tube.</td>
<td>Do not stick pipette tip into the glass fleece when pipetting the DNase.</td>
</tr>
<tr>
<td>Too much starting material</td>
<td>Do not use more than 10⁶ cultured cells, 0.5 ml whole blood, 10⁸ yeast, or 10⁹ bacteria.</td>
<td></td>
</tr>
</tbody>
</table>

VII.  Factors that may affect the High Pure RNA Tissue Kit

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low nucleic acid yield or purity</td>
<td>Tissue stored and handled in less than optimal conditions</td>
<td>Use fresh tissue and disrupt immediately or flash frozen tissue stored at –60°C or below. Frozen tissue should not be allowed to thaw during handling prior to disruption in Lysis/Binding Buffer.</td>
</tr>
<tr>
<td>Ethanol not added to the lysate</td>
<td>Addition of 0.5 volume of absolute ethanol to the lysate is necessary to promote selective binding of RNA to the glass fibers.</td>
<td></td>
</tr>
<tr>
<td>High levels of RNase activity</td>
<td>Be careful to create an RNase-free working environment.</td>
<td>Process starting material immediately or store it at –80°C until it can be processed.</td>
</tr>
</tbody>
</table>

Tissue homogenate is viscous and difficult to pipette, low RNA yield

| Insufficient disruption or homogenization | Add 350 μl of Lysis/Binding Buffer and repeat homogenization step to reduce viscosity. |
| Too much starting material | Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer. |
### VII. Factors that may affect the High Pure RNA Tissue Kit, continued

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clogged filter tube</td>
<td>Insufficient disruption and/or homogenization</td>
<td>• E.g., increase the disruption time for the rotor stator homogenizer or pass through syringe/needle several times.</td>
</tr>
<tr>
<td></td>
<td>Too much starting material</td>
<td>• Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer.</td>
</tr>
<tr>
<td>DNA contamination</td>
<td>Lysis/Binding Buffer not completely removed from the glass fleece</td>
<td>• Increase centrifugation time.</td>
</tr>
</tbody>
</table>

### VIII. Factors that may affect the High Pure FFPE RNA Micro Kit

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low RNA yield or purity</td>
<td>High levels of RNase activity</td>
<td>• Be careful to create an RNase-free working environment.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Process starting material immediately or store it at –80°C until it can be processed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Use eluted RNA directly in downstream procedures or store it immediately at –80°C.</td>
</tr>
<tr>
<td>Tissue homogenate is viscous and difficult to pipette, low RNA yield</td>
<td>Insufficient disruption or homogenization</td>
<td>• Add 350 μl of Lysis/Binding Buffer and repeat homogenization step to reduce viscosity.</td>
</tr>
<tr>
<td>Low yield and/or bad performance in RT-PCR</td>
<td>Nucleic acid is cross-linked to impurities</td>
<td>• Increase Proteinase K digestion time in step 1 of the RNA Isolation Protocol to 16 h (overnight).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer.</td>
</tr>
<tr>
<td>Clogged filter tube</td>
<td>Insufficient disruption and/or homogenization</td>
<td>• E.g., increase the disruption time for the rotor stator homogenizer or pass through syringe/needle several times.</td>
</tr>
<tr>
<td></td>
<td>Too much starting material</td>
<td>• Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer.</td>
</tr>
</tbody>
</table>
### IX. Factors that may affect the High Pure RNA Paraffin Kit

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low RNA yield and purity</td>
<td>Inappropriate storage and handling of tissue</td>
<td>▶ Use fresh tissue and disrupt immediately or flash-frozen tissue stored at –70°C. Frozen tissue should not be allowed to thaw during handling prior to disruption in Lysis/Binding Buffer.</td>
</tr>
<tr>
<td>Tissue homogenate is viscous and difficult to pipette</td>
<td>Insufficient disruption or homogenization</td>
<td>▶ Add 350 μl of Lysis/Binding Buffer and repeat homogenization step to reduce viscosity.</td>
</tr>
<tr>
<td></td>
<td>Too much starting material</td>
<td>▶ Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer.</td>
</tr>
<tr>
<td>Clogged filter tube</td>
<td>Insufficient disruption and/or homogenization</td>
<td>▶ Increase the disruption time for e.g., the rotor stator homogenizer or pass through syringe/needle several times.</td>
</tr>
<tr>
<td></td>
<td>Too much starting material</td>
<td>▶ Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer.</td>
</tr>
</tbody>
</table>

### X. Factors that may affect the High Pure Viral RNA Kit

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low RNA yield</td>
<td>High levels of RNase activity</td>
<td>▶ See suggestions under “No RNA” in “Factors that may affect the High Pure RNA Isolation Kit” page 102.</td>
</tr>
<tr>
<td>Carrier RNA not completely dissolved</td>
<td>To solubilize the lyophilized Poly(A) Carrier RNA completely:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>▶ 1. Pipette 0.4 ml Elution Buffer into the glass vial containing lyophilized Carrier RNA.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>▶ 2. Stopper and invert the vial until all the lyophilize (including any stuck to the rubber stopper) is dissolved.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>▶ 3. Aliquot the carrier RNA mark each aliquot with the date of reconstitution, and store at –15 to –25°C.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>❗ Thaw one vial with 4 μl carrier RNA and mix thoroughly with 5 ml Binding Buffer.</td>
<td></td>
</tr>
</tbody>
</table>
XI. Factors that may affect the High Pure Viral Nucleic Acid Kit or the High Pure Viral Nucleic Acid Kit Large Volume

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low RNA yield</td>
<td>High levels of RNase activity</td>
<td>See suggestions under “No RNA” in “Factors that may affect the High Pure RNA Isolation Kit” page 102.</td>
</tr>
</tbody>
</table>
| Low DNA or RNA yield   | Carrier RNA not completely dissolved       | To solubilize the lyophilized Poly(A) Carrier RNA completely:  
  1. Pipette 0.5 ml Elution Buffer into the glass vial containing lyophilized Carrier RNA.  
  2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.  
  3. Aliquot the Carrier RNA, mark each aliquot with the date of reconstitution, and store at –15 to –25°C.  
  4. **Thaw one vial with 50 μl carrier RNA and mix thoroughly with 2.5 ml Binding Buffer.** |
| Incomplete Proteinase K digestion |                                   | Be sure to dissolve the lyophilized Proteinase K completely, as follows:  
  1. Pipette 5 ml of PCR grade water into the glass vial containing lyophilized Proteinase K.  
  2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.  
  3. Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at –15 to –25°C.  
  4. **Reconstituted Proteinase K is stable for 12 months when stored properly.** |
## Agarose Gel DNA Extraction Kit

for recovery of up to 100 DNA fragments from agarose gel slices

Cat. No. 11 696 505 001

### Principle

A solubilization buffer that contains a chaotropic salt (sodium perchlorate) dissolves an agarose gel slice that contains a DNA fragment. In the presence of the chaotrope, the DNA fragment binds selectively to silica matrix. The DNA remains bound while a series of rapid “wash-and-spin” steps remove contaminating small molecules. Finally, low salt elution removes the DNA from the silica matrix. The process does not require DNA precipitation, organic solvent extractions, or extensive handling of the DNA.

### Starting material

100 – 200 mg agarose gel slices (≤2% agarose) containing:

- Double-stranded product DNA (0.4 – 100 kb) from PCR or other enzymatic reactions
- Single-stranded oligonucleotides (>20 nucleotides)

**Kit contains enough reagents for purification of 100 DNA fragments from gel slices containing up to 2% agarose. Fewer DNA fragments can be purified from higher percentage gels, since recovery of DNA from gel slices containing >2% agarose requires twice the standard amount of solubilization buffer.**

### Application

- Preparation of concentrated, purified DNA, which may be used directly for labeling, sequencing, cloning, PCR, and other procedures requiring concentrated DNA

### Time required

- Total time: 60 min (+ gel running time)
- Hands-on time: <30 min

### Results

- Recovery:
  - DNA fragments (0.4 – 9.5 kb long), approx. 80%.
  - Large DNA fragments (10 – 100 kb), approx. 60%.
  - Single-stranded oligonucleotides (>20 bases), approx. 65%.
  - Purity: Purified DNA is free of agarose and all inhibitors of downstream procedures (small molecules, nucleotides, proteins).

### Benefits

- **Saves time**, since DNA extracted with the kit can be used directly in most common procedures that require concentrated DNA (restriction enzyme digestion, enzymatic labeling, cloning, etc.).
- **Minimizes DNA loss**, because the kit recovers DNA from agarose without solvent extraction, precipitation, or other steps that can lead to lost or degraded DNA.
- **Minimizes shearing of large DNA**, since the kit requires only minimal handling of DNA.
- **Increases lab safety**, because the kit does not use hazardous organic solvents.
How to use the kit

I. Flow diagram

1. Separate DNA electrophoretically in an agarose gel
2. Cut gel slice containing DNA
3. Solubilize gel slice and bind DNA to Silica Matrix
4. Pellet Silica Matrix and discard the supernatant
5. Wash Silica Matrix (3 x), pelleting after each wash
6. Drain residual liquid from Silica Matrix
7. Elute DNA from the Silica Matrix

II. Kit contents

- Silica Matrix, suspension containing sodium perchlorate
- Agarose Gel Solubilization Buffer with sodium perchlorate (60 ml)
- Nucleic Acid Binding Buffer with sodium perchlorate (100 ml)
- Wash Buffer (20 ml)

⚠ Add 80 ml absolute ethanol to Wash Buffer before use

III. Additional materials needed

- Agarose LE, MP or MS (Roche Applied Science)
- Gel running buffer:
  - TAE Buffer (40 mM Tris-acetate, 1 mM EDTA), pH 8.0 – 8.5
  - TBE Buffer (45 mM Tris-borate, 1 mM EDTA), pH 8.0 – 8.5
- Electrophoresis equipment
- Sterile scalpel
IV. Protocol for preparing DNA from agarose gels

? Gels containing >2% agarose may be solubilized with twice the standard amount of Solubilization Buffer. For details, see the package insert supplied with the kit.

1. Separate the DNA of interest (for example, PCR product) electrophoretically:
   - Prepare an agarose gel with Roche Applied Science agarose.
   - Load PCR product on the gel along with a DNA molecular weight marker of known size and concentration.
   - Use 1 x TAE or 1 x TBE as running buffer.
   - Electrophorese until PCR product is well separated from contaminants.

2. After gel electrophoresis:
   - Stain the gel with ethidium bromide.
   - Estimate the concentration of the PCR product by comparing its intensity with that of a DNA standard of similar size and known concentration.

3. Cut the DNA band from the gel with a sharp scalpel or razor blade.
   - Cut the smallest possible gel slice.

4. Place the agarose gel slice into a sterile microcentrifuge tube (1.5 – 2.0 ml) of known weight, then reweigh the tube.

5. Add 300 μl Agarose Gel Solubilization Buffer to each 100 mg of agarose gel.

6. Resuspend the Silica Matrix to produce a homogeneous suspension. Then, depending on the amount of DNA in the gel slice, do one of the following:
   - If the gel slice contains ≤2.5 μg DNA, add 10 μl of the resuspended Silica Matrix to the agarose gel slice.
   - If the gel slice contains >2.5 μg DNA, add 4 μl of the resuspended Silica Matrix to the agarose gel slice for every μg of DNA in the gel.

7. To dissolve the agarose gel slice and bind the DNA to the Silica Matrix:
   - Vortex the microcentrifuge tube briefly to resuspend the gel slice in the Solubilization Buffer.
   - Incubate the suspension for 10 min at +56 to +60°C.
   - Vortex the tube briefly every 2 – 3 min during the incubation.

8. Pellet the Silica Matrix (which contains the DNA) by centrifuging the tube in a tabletop microcentrifuge at maximum speed for 30 s. Discard the supernatant.

9. Resuspend the Silica Matrix by adding 500 μl of Nucleic Acid Binding Buffer and vortexing.

10. Repeat step 8.

11. Wash the Silica Matrix by adding 500 μl of Wash Buffer and vortexing.

12. Repeat step 8.

13. Repeat the wash steps (Steps 11 and 12).
1. Remove remaining supernatant with a Pasteur pipette, then invert tube over a sterile absorbent tissue and let silica pellet dry for 15 min at +15 to +25°C.  
   **Do not dry the pellet under vacuum, since overdrying will reduce the amount of DNA recovered from the matrix.**

2. To elute the DNA from the Silica Matrix:
   - To the pellet, add 20 – 50 μl of TE Buffer or PCR grade water (adjusted to pH 8.0 – 8.5 before use).
   - Resuspend the pellet by vortexing.
   - Incubate the tube for:
     - EITHER 10 min at +15 to +25°C
     - OR 5 min at +56 to +60°C
   - For large DNA fragments (>9.5 kb), incubate at +56° to +60°C for 15 – 20 min.
   - Vortex the tube briefly every 2 – 3 min to resuspend the Silica Matrix.

3. After the elution is complete:
   - Pellet the Silica Matrix by centrifuging the tube in a microcentrifuge at maximum speed for 30 s.
   - Transfer the supernatant (which contains the DNA) to a new sterile microcentrifuge tube.

   **Avoid transferring any Silica Matrix with the supernatant.**

---

**V. Troubleshooting the Agarose Extraction protocol**

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low yield of DNA</td>
<td>Agarose gel not completely solubilized</td>
<td>Be sure that the incubation temperature in Step 7 is at least +56°C.</td>
</tr>
<tr>
<td></td>
<td>Ethanol from the Wash Buffer remains during DNA elution</td>
<td>Before eluting the DNA, completely remove Wash Buffer with a Pasteur pipette, then air dry the pellet thoroughly.</td>
</tr>
<tr>
<td></td>
<td>Elution Buffer has acidic pH</td>
<td>Check that pH of the Elution Buffer is 8.0 – 8.5.</td>
</tr>
<tr>
<td></td>
<td>Large DNA fragments adsorbed strongly to Silica Matrix</td>
<td>Perform elution (Step 16) at +56 to +60°C for 15 – 20 min.</td>
</tr>
</tbody>
</table>
| | Pellet is too dry | Resuspend the pellet in TE Buffer and incubate at +56 to +60°C for 10 min.  
   **Vortex the tube every 2 – 3 min during incubation.** |
V. Troubleshooting the Agarose Extraction protocol, continued

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of subsequent enzymatic reactions by recovered PCR product</td>
<td>Ethanol from the Wash Buffer remains during DNA elution</td>
<td>◄ Before eluting the DNA, completely remove Wash Buffer with a Pasteur pipette, then air dry the pellet thoroughly.</td>
</tr>
<tr>
<td></td>
<td>Silica Matrix not completely removed from the eluted DNA</td>
<td>◄ Recentrifuge DNA solution and transfer supernatant to a new microcentrifuge tube.</td>
</tr>
<tr>
<td></td>
<td>Nucleic Acid Binding Buffer not completely removed and chaotropic salts inhibit reaction.</td>
<td>◄ Remove Binding Buffer completely.</td>
</tr>
</tbody>
</table>

Typical results with the kit

Figure 37: Recovery of DNA fragments from agarose gels with the Agarose Gel DNA Extraction Kit.
DNA fragments (0.5 – 23 kb) from the Roche Applied Science Molecular Weight Marker II (MWM II) were separated on a 1% agarose gel, extracted from the gel, and isolated according to the protocol given in the text. Isolated fragments were displayed on a new agarose gel.

Lanes 1, 9: MWM II
Lane 2: 23 kb fragment
Lane 3: 9.5 kb fragment
Lane 4: 6.5 kb fragment
Lane 5: 4.3 kb fragment
Lane 6: 2.3 kb fragment
Lane 7: 2.0 kb fragment
Lane 8: 0.5 kb fragment

Result: The Agarose Gel DNA Extraction Kit isolated good yields of both large and small DNA fragments.

References

High Pure 96 UF Cleanup Kit

for high-throughput purification of PCR products by ultrafiltration

Cat. No. 04 422 694 001 (2 x 96 purifications)

**Principle**
The kit uses ultrafiltration and size exclusion to separate small from large molecules. Small contaminants (dNTP's, primer, primer dimers, salts, etc.) that may interfere with downstream applications readily pass through a membrane (and into a waste container), while PCR products remain atop the membrane. The purified DNA can either be washed to ensure complete removal of contaminants or resuspended in buffer for immediate use in downstream procedures.

**Starting material**
The kit can recover 20 - 300 μl PCR products that range from 100 bp to >10 kb.

**Application**
Use the High Pure 96 UF Cleanup Kit to quickly remove dNTPs, salts, primers and other small molecules from your PCR products, making them suitable for: microarray spotting, labeling, restriction digest, automated fluorescent sequencing, and cloning.

**Time required**
- Manual processing on vacuum manifolds or microplate centrifuges takes approx. 20 min for 96 samples. Automated processing depends on the liquid handling instrument used; it can be as little as 15 min.

**Results**
- The purity of the recovered DNA is shown by its $A_{260}/A_{280}$ ratio, which is 1.8 ± 0. The amount of DNA recovered depends on the elution volume and the length of the PCR product. Typically, 90% of a 1 kb fragment can be recovered in 25 μl elution buffer. The recovery rate depends on the length of the amplification product: ≥ 150 bp recovery ≥ 40%; 1500 bp to 4500 bp recovery ≥ 90%; and 8000 bp recovery ≥ 80%.

**Benefits**
- **Flexible:** works well with vacuum manifolds, microplate centrifuges, and common liquid handling instruments.
- **Simple:** provides all necessary components in ready-to-use form.
- **Suitable for high volume laboratories:** purifies up to 96 samples in less than 20 minutes.
- **Avoids cross-contamination:** isolates samples in 96 individual wells to ensure that no well-to-well or aerosol „cross-talk“ occurs.
- **Convenient:** samples are applied and removed from the top.
- **Efficient:** gives excellent recovery – even with small (100 bp) PCR fragments.
How to use the kit

I. Flow diagram

Using Vacuum Manifold

Transfer PCR Sample mix (20 to 300 µl) to High Pure 96 UF Cleanup Plate

Apply vacuum (-400 mbar to -600 mbar) for 10 min or until all liquid has passed through membrane and membrane appears dry

Vent vacuum manifold

Apply vacuum (-400 mbar to -600 mbar) for 10 min or until all Wash Buffer has passed through membrane and membrane appears dry

Vent vacuum manifold

Incubate for 10 to 15 min or Pipette up and down 10 times or Shake moderately 2 to 5 min

Transfer resuspended PCR product to Elution Plate

Purified PCR product

II. Kit content

- 2 High Pure 96 UF Cleanup Plates
- 100 ml Wash Buffer
- 2 Waste Plates
- 100 ml Resuspension Buffer
- 2 Elution Plates with self-adhering foil
III. Additional materials needed

- Vacuum manifold OR
- Microtiter plate centrifuge capable of 4500 x g centrifugal force (e.g., Beckman Coulter Allegra 25R)
- Multichannel pipette (optional)

Schematic overview of PCR product purification

IV. Isolation Protocol

IVA. Isolation Protocol Using a Vacuum Manifold

The protocol is designed for reaction volumes of 20 to 100 μl. You will have to increase the filtration time to process larger volumes (up to 300 μl). This manual isolation protocol may be automated for use with common liquid handling systems.

1. Pipette 20 to 100 μl PCR reaction mix directly onto the membrane in one well of the High Pure 96 Cleanup Plate.
   - Avoid dispensing sample onto the inner wall of the well. Unused wells of the plate may be left open. They do not need to be sealed.

2. Remove contaminants by ultrafiltration. Place plate on a suitable vacuum manifold (such as those listed in the package insert) and apply vacuum until all liquid has passed through the membrane.
   - Indicated times are approximate. Depending on the sample, the time required may increase or decrease. Make sure that all liquid has passed through the membrane (membrane will appear dry). After all liquid has been filtered, dry the membrane for an additional 30–60 s by continuing to apply vacuum. 400 to 600 mbar for 10 to 15 min.
How to use the kit

V. High Pure 96 UF Cleanup Kit

II. How to use the kit

Optional wash step: Vent the vacuum manifold for 60 to 90 s. Dispense 100 μl Wash Buffer into each well. Apply vacuum 400 to 600 mbar for 10 to 15 min to draw liquid through the membrane until all Wash Buffer has passed through. Apply vacuum for an additional 30–60 s.

Typically, you do not need to wash the collected DNA. However, if you have problems with downstream applications or want to ensure removal of small molecules, we recommend using the extra wash step.

Vent the vacuum manifold for 60 to 90 s. Add 25 to 100 μl Resuspension Buffer directly to the center of the membrane in each well.

* The dead volume of the membrane is 3–4 μl

Incubate the Resuspension Buffer on the membrane for 5–10 min OR resuspend DNA by pipetting the liquid up and down 5–10 times mixing OR resuspend DNA by placing the Cleanup Plate on a microplate shaker for 2 to 5 min. (We recommend using 50 μl Resuspension Buffer for resuspension on a shaker).

Use a pipette to remove the resuspended, purified product from Cleanup Plate and transfer it to a clean Elution Plate.

Use purified product directly in downstream applications or seal Elution Plate with foil for storage of the resuspended DNA samples.

VIb. Isolation Protocol Using a Microtiter Plate Centrifuge

1. Pipette 20 to 100 μl PCR reaction mix directly onto the membrane in one well of the High Pure 96 UF Cleanup Plate.

   * Avoid dispensing sample onto the inner wall of the well. Unused wells of the plate may be left open. They do not need to be sealed.

2. Place the plate onto the Waste Collection Plate (supplied with the kit). Place the plate „sandwich“ in a suitable centrifuge (such as those listed in package insert) and spin at 4,500 x $g$ for 5–10 min at $+15$ to $+25^\circ$C.

   * Indicated times are approximate. Depending on the sample, the time required may increase or decrease. Make sure that all liquid has passed through the membrane (membrane will appear dry).

3. Wash the membrane by adding 100 μl Wash Buffer directly to the center of the membrane in each well (without separating the Cleanup Plate/Waste Plate sandwich). Centrifuge the assembly at 4,500 x $g$ for 5–10 min at $+15$ to $+25^\circ$C.

   * We strongly recommend performing the wash step when you are using centrifugation to purify the DNA. After the first spin, minute amounts of liquid (containing salts etc.) will remain on top of the membrane and contaminate the resuspended sample.

4. Add 25 to 100 μl Resuspension Buffer directly to the center of the membrane in each well.

   * The dead volume of the membrane is 3–4 μl

5. Incubate the Resuspension Buffer on the membrane for 5–10 min OR resuspend DNA by pipetting the liquid up and down 5–10 times mixing OR resuspend DNA by placing the Cleanup Plate on a microplate shaker for 2 to 5 min. (We recommend using 50 μl Resuspension Buffer for resuspension on a shaker).

6. Use a pipette to remove the resuspended, purified product from the Cleanup Plate and transfer it to the clean Elution Plate.

7. Use purified product directly in downstream applications or seal Elution Plate with foil for storage of the resuspended DNA samples.
V. Factors that may affect the High Pure 96 UF Cleanup Kit

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low DNA recovery</td>
<td>Elution conditions not optimal</td>
<td>► Increase number of mixing steps.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>► Increase incubation time.</td>
</tr>
<tr>
<td>Did not use enough Resuspension Buffer</td>
<td>Recommended amount of Resuspension Buffer: at least 25 μl for manual purification or at least 50 μl for automated purification or resuspension on a shaker</td>
<td></td>
</tr>
<tr>
<td>DNA fragments dried on membrane</td>
<td>Dispense Resuspension Buffer or PCR grade water onto membrane and incubate 15 to 30 min at +15 to +25°C to allow DNA to rehydrate before trying to recover it.</td>
<td></td>
</tr>
<tr>
<td>Small PCR Product</td>
<td></td>
<td>► Increase number of mixing/resuspension steps.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>► Pipette the resuspended DNA up and down at least 5-10 times.</td>
</tr>
<tr>
<td>Contaminated samples</td>
<td>Samples not filtered completely</td>
<td>► Force samples completely through filter until membrane appears dry and shiny</td>
</tr>
<tr>
<td>Sample remains on the inner wall of well</td>
<td></td>
<td>► Dispense samples directly onto the membrane. Make sure that no sample material sticks to the side of the wells.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>► Avoid letting the pipette tip touch the wall during automated purification.</td>
</tr>
<tr>
<td>Slow filtration</td>
<td>PCR buffer contains detergents</td>
<td>► Although detergents are effectively removed by ultrafiltration on the High Pure 96 UF Cleanup Plate, the presence of detergent in commercially available PCR buffers may lead to increased filtration time.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>► Use of detergent-free PCR buffers is recommended.</td>
</tr>
</tbody>
</table>
Typical results with the kit

Figure 38: Purification of small and large PCR products with the High Pure 96 UF Cleanup Kit and competing products. Equal amounts of sample were analyzed on a gel after they were purified with the indicated products. Lanes 1: 1.7 kb PCR fragment, lanes 2: 1.2 kb PCR fragment, lanes 3: 600 bp PCR fragment, lanes 4: 350 bp PCR fragment, lanes 5: 165 bp PCR fragment. (M: DNA Molecular Weight marker XIV.)

Figure 39: Comparison of purification via vacuum (lanes 7-12) and centrifugation (lanes 1-6, 13-18). Equal amounts of sample were analyzed on a gel after they were purified with the High Pure UF 96 Cleanup Kit using the indicated method. Samples analyzed in lanes 1-6 and 13-18 were purified on different High Pure 96 UF Cleanup Plates. Lane marked „Input“ contained a sample of the PCR product obtained before purification. (M: DNA Molecular Weight Marker XIV.)

Reference

Overview of Ion Exchange Chromatography 134
Genopure Plasmid Midi Kit 136
Genopure Plasmid Maxi Kit 143
Genopure Buffer Set for Low-Copy Number Plasmids 150
Overview of Ion Exchange Chromatography

This chapter describes our kits for midi and maxi preparation of ultrapure plasmid DNA.

The method:
- Eliminates CsCl gradient ultracentrifugation which requires days.
- Avoids use of phenol/chloroform extraction
- Uses gravity flow columns, no centrifugation required

For a general overview of these products, continue reading this article, or for detailed information of the single product turn to the page which describes the product in detail.

<table>
<thead>
<tr>
<th>If you are interested in</th>
<th>For preparing</th>
<th>See page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genopure Plasmid Midi Kit</td>
<td>Plasmid DNA from 5 to 30 ml bacterial culture suitable for transfection, PCR, restriction analysis/Southern blotting, sequencing, cloning</td>
<td>136</td>
</tr>
<tr>
<td>Genopure Plasmid Maxi Kit</td>
<td>Plasmid DNA from 30 to 150 ml bacterial culture for transfection, PCR, restriction analysis/Southern blotting, sequencing, cloning</td>
<td>143</td>
</tr>
<tr>
<td>Genopure Buffer Set</td>
<td>For isolation of low-copy number plasmid DNA in combination with the Genopure Plasmid Kits.</td>
<td>150</td>
</tr>
</tbody>
</table>

The isolation method is based on a modified alkaline lysis protocol and can be divided into the following steps:
- Harvest and disruption of the bacterial cells
- Precipitation of the bacterial "chromosomal" DNA
- Clarification of the bacterial lysate
- Adsorption of the plasmid DNA to the column matrix
- Removal of residual impurities by wash steps
- Elution of the plasmid DNA by high salt conditions
- Concentration and salt removal by alcohol precipitation

The isolation method is optimized for cultures grown in LB media; other rich media may require increased volumes of Suspension-, Lysis or Neutralization Buffer, and an additional wash step.

The isolation procedure is suitable for all plasmid sizes; lysates of larger constructs (up to 100 kb) should be cleared by filtration to avoid shearing.

The yield of plasmid DNA preparations is dependent on several parameters, e.g., quality of the bacterial culture growth, amount of used culture suspension for the preparation, plasmid type used etc.

As a general rule the typical yield of a high copy number plasmid is about 3 – 5 μg of DNA per ml of original bacterial culture (pUC, pTZ, pGEM in common host strains as XL-1 blue, HB101, JM 109).

The typical yield of low copy number plasmids is about 0.2 – 1 μg of DNA per ml of original bacterial culture. It is recommended to use the supplementary Genopure Buffer Set for low copy number plasmids in combination with the respective Genopure Plasmid Kit.
Both kits are supplied with folded filters to eliminate the time-consuming centrifugation step after the alkaline lysis. In approximately 2 min (midi) or 10 min (maxi), respectively, of unattended running cellular debris and potassium dodecyl sulfate precipitates are held back by the filter thereby avoiding shearing of large DNA constructs. Besides the significant reduction of preparation time another advantage of filtration is that even small SDS precipitates which cannot be separated by conventional centrifugation are completely removed.

Figure 40: Genopure plasmid purification procedures. Schematic representation of clearing the lysate by filtration with subsequent plasmid purification.
## Genopure Plasmid Midi Kit

for up to 20 preparations of plasmid DNA in medium scale  
Cat. No. 03 143 414 001

| Principle | The isolation procedure is based on a modified alkaline lysis protocol and can be divided into the following steps:  
The bacteria are partially lysed, allowing the plasmid DNA to escape the cell wall into the supernatant. The larger *E. coli* chromosomal DNA is trapped in the cell wall. The lysate is cleared of cellular debris and the plasmid DNA containing fraction is added to the column. The bound plasmid DNA is washed to remove contaminating bacterial components. The plasmid DNA is eluted and precipitated to remove salt and to concentrate the eluate.  
This is a commonly used method that generates highly purified plasmid DNA (free of RNA contamination). |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting material</td>
<td></td>
</tr>
</tbody>
</table>
- 5 – 30 ml *E. coli* cultures transformed with a high copy number plasmid.  
- 10 – 100 ml *E. coli* cultures transformed with a low copy number plasmid  
(at a density of 2 – 6.0 A₆₀₀ units per ml bacterial culture). |
| Application | This kit is used to prepare plasmid DNA in medium quantities known as "midi preps". Using a modified alkaline lysis method highly purified plasmid DNA is generated. The kit is designed for the isolation of up to 100 μg of plasmid DNA from bacterial culture.  
Depending on the copy number of the plasmids use either 5 to 30 ml (high copy number) or 10 to 100 ml (low copy number) bacterial suspension. The quality of the plasmid DNA is better than plasmid DNA obtained by 2 x CsCl gradient centrifugation. As a result, the plasmid DNA is suitable for all molecular biology applications e.g., transfection, PCR, restriction analysis/Southern blotting, sequencing and cloning. |
| Time required |  
- Total time: 60 min including a filtration step after the alkaline lysis.  
- Hands-on time: Minimal hands-on time required (about 10 min). |
| Results |  
- Purity: Plasmid DNA is ready-to-use even in demanding down-stream applications.  
- Yield: Depending on *E. coli* strain and density of the cell culture. Comparable to traditional purification methods.  
- Application: The purified plasmid has been used for PCR, sequencing and transfection with excellent results. |
| Benefits |  
- **Avoids organic or toxic materials** as no phenol or chloroform, CsCl and ethidium bromide needed.  
- **Ready to use** because all reagents provided with the kit.  
- **Reliable quality** because better than 2 x CsCl.  
- **Parallel processing** because of use of high speed gravity flow columns.  
- **All plasmid sizes** can be isolated even BAC DNA. |
How to use the kit

I. Flow diagram

Discard supernatant

Centrifuge 5 – 30 ml E. coli culture, at 3000 – 5000 x g at +2 to +8°C for 15 min

Resuspend pellet in 4 ml Suspension Buffer/RNase

Add 4 ml Wash Buffer

Add 5 ml Elution Buffer

Add 3 ml chilled 70% ethanol

Add 20 – 100 μl TE Buffer or PCR grade water

MIX gently by inversion, incubate 5 min on ice

Mix gently, incubate at +15 to +25°C 2 – 3 min

Add 4 ml Lysis Buffer

Add 4 ml chilled Neutralization Buffer

Mix gently by inversion, incubate 5 min on ice

Clear bacterial lysate by filtration (or by centrifugation), transfer flowthrough (or supernatant) to pre-equilibrated column

Discard pellet

Allow the column to empty by gravity flow and repeat this wash step

Add 5 ml Wash Buffer

Add 5 ml Lysis Buffer

Add 4 ml chilled 70% ethanol

Allow the column to empty by gravity flow and collect flowthrough

Discard flowthrough

Centrifuge at 15,000 x g at +2 to +8°C for 30 min

Add 3.6 ml isopropanol equilibrated to +15 to +25°C

Discard supernatant

Centrifuge at 15,000 x g at +2 to +8°C for 10 min

Briefly air-dry the pellet (10 min) and redissolve the pellet

Discard supernatant

Mix gently, incubate at +15 to +25°C 2 – 3 min

Add 5 ml Wash Buffer

Add 5 ml Elution Buffer

Add 3 ml chilled 70% ethanol

Add 20 – 100 μl TE Buffer or PCR grade water

Discard supernatant
II. Kit contents

- Suspension Buffer (100 ml) for suspension of bacterial cell pellets
- RNase A (12 mg) for dissolution in Suspension Buffer
- Lysis Buffer (100 ml) for bacterial cell lysis
- Neutralization Buffer (100 ml) to form a stable cellular debris precipitate
- Equilibration Buffer (70 ml) for equilibrating the columns prior to use
- Wash Buffer (250 ml) for removal of residual impurities
- Elution Buffer (125 ml) for plasmid elution
- NucleoBond AX 100 Columns (20 columns) for the isolation step
- Folded filters (20 filters) to eliminate a centrifugation step and to remove cellular debris
- Sealing rings (10 rings) to station the columns in test tubes

III. Additional materials needed

- Centrifuge and tubes for harvesting bacterial cultures, capable of ≥15,000 x g
- Isopropanol
- 70% ethanol
- TE Buffer or other low salt buffer
- Tube for collecting and precipitating eluted plasmid DNA
- Funnel for clearing of lysates by folded filters
IV. Protocol for preparing high copy number plasmid DNA

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
<th>Time / x g / Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Centrifuge bacterial cells from 5 – 30 ml <em>E. coli</em> culture grown in LB medium. Discard the supernatant. Carefully resuspend the pellet in 4 ml Suspension Buffer + RNase and mix well.</td>
<td>5 – 10 min / 3000 – 5000 x g / +2 to +8°C</td>
</tr>
<tr>
<td>2</td>
<td>Add 4 ml Lysis Buffer to the suspension and mix gently by inverting the tube 6 to 8 times and incubate. <em>Do not vortex in order to avoid shearing and release of genomic DNA. Do not incubate for more than 5 min to prevent the release of chromosomal DNA from the cell debris.</em></td>
<td>2 – 3 min at +15 to +25°C</td>
</tr>
<tr>
<td>2</td>
<td>Add 4 ml chilled Neutralization Buffer to the suspension. Immediately mix the suspension gently by inverting the tube 6 to 8 times until a homogeneous suspension is formed. Incubate the tube. <em>The solution becomes cloudy and flocculent precipitate will form.</em></td>
<td>5 min on ice</td>
</tr>
<tr>
<td>3</td>
<td>Clear the lysate by either centrifugation (4a) or by filtration (4b).</td>
<td>&gt;30 min / &gt;12,000 x g / +2 to +8°C</td>
</tr>
<tr>
<td>4</td>
<td>Centrifuge at high speed Directly after centrifugation carefully remove the supernatant from the white precipitate and proceed with step 5.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Put a folded filter into a funnel inserted in a 50 ml plastic tube. Moisten the filter with a few drops of Equilibration Buffer or PCR grade water. Load the lysate onto the wet folded filter and collect the flowthrough. <em>The SDS is removed with the Neutralization Buffer (white precipitate) and should not be loaded onto the column. If the supernatant is not clear, load it again onto a folded filter to prevent clogging of the column.</em></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Mount the sealing ring on the column as shown in Figure 41 to fix the column in the Collection Tube. Insert one column into one Collection Tube. Equilibrate the column with 2.5 ml Equilibration Buffer. Allow the column to empty by gravity flow. Discard the flowthrough.</td>
<td>Figure 41</td>
</tr>
<tr>
<td>7</td>
<td>Load the cleared lysate of step 4 onto the equilibrated column. Allow the column to empty by gravity flow. Discard the flowthrough.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Wash the column with 5 ml Wash Buffer. Allow the column to empty by gravity flow. Discard the flowthrough.</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Repeat step 7. Discard flowthrough and Collection Tube.</td>
<td></td>
</tr>
</tbody>
</table>
IV. Protocol for preparing high copy number plasmid DNA, continued

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
<th>Time / x g / Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Re-insert the column into a new Collection Tube capable of withstanding high speed centrifugation (≥15,000 x g). Elute the plasmid with 5 ml Elution Buffer. Allow the column to empty by gravity flow. The collected flowthrough contains the plasmid.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Precipitate the eluted plasmid DNA with 3.6 ml isopropanol equilibrated to +15 to +25°C. Centrifuge immediately at high speed. Carefully discard the supernatant.</td>
<td>30 min / ≥15,000 x g / +2 to +8°C</td>
</tr>
<tr>
<td>11</td>
<td>Wash the plasmid DNA with 3 ml chilled 70% ethanol. Centrifuge at high speed. Carefully remove ethanol from the tube with pipette tip. Air-dry the plasmid DNA pellet.</td>
<td>+2 to +8°C 10 min / &gt;15,000 x g / +2 to +8°C</td>
</tr>
<tr>
<td>12</td>
<td>Carefully redissolve the plasmid DNA pellet in 20 – 100 μl TE Buffer or PCR grade water.</td>
<td>10 min</td>
</tr>
</tbody>
</table>

V. Troubleshooting the Genopure Plasmid Midi protocol

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low nucleic acid yield or purity</td>
<td>Kit stored under non-optimal conditions</td>
<td>Store kit at +15 to +25°C at all times upon arrival.</td>
</tr>
<tr>
<td>Buffers or other reagents were exposed to conditions that reduced their effectiveness</td>
<td></td>
<td>Store all buffers at +15 to +25°C. After reconstitution of RNase with Suspension Buffer store aliquots at +2 to +8°C. Close all reagent bottles tightly after each use to preserve pH, stability and freedom from contamination.</td>
</tr>
<tr>
<td>Reagents and samples not completely mixed</td>
<td></td>
<td>Always mix the sample tube well after addition of each reagent. Ensure Lysis Buffer and Neutralization Buffer are free of precipitates.</td>
</tr>
<tr>
<td>Low recovery of nucleic acids after elution</td>
<td>Non-optimal reagent has been used for elution. Salt is required for optimal elution</td>
<td>Use the Elution Buffer of the kit.</td>
</tr>
</tbody>
</table>
V. Troubleshooting the Genopure Plasmid Midi protocol, continued

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low plasmid yield</td>
<td>Too few cells in starting material</td>
<td>- Grow <em>E. coli</em> to an absorbency ( A_{600} ) of 2 – 6 before harvest.</td>
</tr>
<tr>
<td>Incomplete cell lysis</td>
<td></td>
<td>- Ensure the <em>E. coli</em> pellet is completely resuspended in Suspension Buffer.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Make sure the lysate is clear and viscous after the lysis step (incubation with Lysis Buffer).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Ensure a cloudy white precipitate forms when Binding Buffer is added to the lysate.</td>
</tr>
<tr>
<td>Lysate did not bind</td>
<td></td>
<td>- Pre-equilibrate the column by adding Equilibration Buffer before adding sample.</td>
</tr>
<tr>
<td>completely to column</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA is present in final product</td>
<td>RNase not completely dissolved</td>
<td>- To reconstitute the lyophilized RNase completely:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1. Pipette 1 ml of Suspension Buffer into the glass vial containing lyophilized RNase.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Transfer all the reconstituted RNase back into the Suspension Buffer and mix thoroughly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Mark the reconstituted mixture (enzyme and buffer) with the date of reconstitution and store at +2 to +8°C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Reconstituted mixture is stable for 6 months when stored properly.</strong></td>
</tr>
<tr>
<td>Genomic DNA present in final product</td>
<td>Genomic DNA sheared during lysis step</td>
<td>- Vortexing the preparation after addition of Lysis Buffer should be avoided.</td>
</tr>
<tr>
<td>RNase present in final product</td>
<td>RNase not completely dissolved</td>
<td>- See suggestions under &quot;RNA present in final product&quot; above.</td>
</tr>
<tr>
<td></td>
<td>Too many cells in starting material</td>
<td>- Do not overload the column.</td>
</tr>
<tr>
<td>Additional band running</td>
<td>Denatured plasmid in final product</td>
<td>- Reduce the incubation time during step 2 (lysis step) of the protocol.</td>
</tr>
<tr>
<td>slightly faster than super-coiled plasmid is seen on gels</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Typical results with the kit

Figure 42: The purification process results in contamination-free plasmid DNA.

Table 3: Mean values from several experiments.

<table>
<thead>
<tr>
<th>Plasmid Preparation Method</th>
<th>Endotoxin (EU/μg)</th>
<th>Transfection efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genopure</td>
<td>4 – 10</td>
<td>100</td>
</tr>
<tr>
<td>2-fold CsCl</td>
<td>0.7 – 3</td>
<td>≥ 95</td>
</tr>
<tr>
<td>Alternative Commercial Source (Anion Exchange)</td>
<td>9.3</td>
<td>≥ 80</td>
</tr>
<tr>
<td>Silica-Matrix/Gel Slurry</td>
<td>&gt; 1000</td>
<td>&gt; 30</td>
</tr>
</tbody>
</table>

References


Genopure Plasmid Maxi Kit

for up to 10 preparations of plasmid DNA in large scale
Cat. No. 03 143 422 001

Principle
The isolation procedure is based on a modified alkaline lysis protocol and can be divided into the following steps:
The bacteria are partially lysed, allowing the plasmid DNA to escape the cell wall into the supernatant. The larger E. coli chromosomal DNA is trapped in the cell wall. The lysate is cleared of cellular debris and the plasmid DNA containing fraction is added to the column. The bound plasmid DNA is washed to remove contaminating bacterial components. The plasmid DNA is eluted and precipitated to remove salt and to concentrate the eluate.

This is a commonly used method that generates highly purified plasmid DNA (free of RNA contamination).

Starting material
- 30 – 150 ml E. coli cultures transformed with a high copy number plasmid.
- 100 – 500 ml E. coli cultures transformed with a low copy number plasmid (at a density of 2 – 6.0 A_{600} units per ml bacterial culture).

Application
- This kit is used to prepare plasmid DNA in large quantities known as "maxi preps". Using a modified alkaline lysis method highly purified plasmid DNA is generated. The kit is designed for the isolation of up to 500 μg of plasmid DNA from bacterial culture.
- Depending on the copy number of the plasmids use either 30 to 150 ml (high copy number) or 100 to 500 ml (low copy number) bacterial suspension. The quality of the plasmid DNA is better than plasmid DNA obtained by 2 x CsCl gradient centrifugation.
- Therefore the plasmid DNA is suitable for all molecular biology applications e.g., transfection, PCR, restriction analysis/Southern blotting, sequencing and cloning.

Time required
- Total time: 75 min including a filtration step after the alkaline lysis.
- Hands-on time: Minimal hands-on time required (about 10 min).

Results
- Purity: Plasmid DNA is free of all other bacterial components, including RNA, shown by gel electrophoresis.
- Yield: Depending on E. coli strain and density of the cell culture. Comparable to traditional purification methods.
- Application: The purified plasmid has been used for PCR, sequencing and transfection with excellent results.

Benefits
- **Avoids organic or toxic materials** as no phenol or chloroform, CsCl and ethidium bromide needed.
- **Ready to use** because all reagents provided with the kit.
- **Reliable quality** because better than 2 x CsCl.
- **Parallel processing** because of use of high speed gravity flow columns.
- **All plasmid sizes** can be isolated even BAC DNA.
I. Flow diagram

Centrifuge 30 – 150 ml E. coli culture at 3000 – 5000 x g at +2 to +8°C for 10 min

Mix gently, incubate at +15 to +25°C for 2 – 3 min

Mix gently by inversion, incubate 5 min on ice

Clear bacterial lysate by filtration (or by centrifugation), transfer flowthrough (or supernatant) to pre-equilibrated column

Add 12 ml Wash Buffer

Add 15 ml Elution Buffer

Add 4 ml chilled 70% ethanol

Add 11 ml isopropanol equilibrated to +15 to +25°C

Add 16 ml Wash Buffer

Add 15 ml Elution Buffer

Add 100 – 500 μl TE Buffer or PCR grade water

Discard supernatant

Discard pellet

Discard flowthrough

Discard supernatant

Discard supernatant

Centrifuge at 15,000 x g at +2 to +8°C for 10 min

Centrifuge at 15,000 x g at +2 to +8°C for 30 min

Allow the column to empty by gravity flow and collect flowthrough

Allow the column to empty by gravity flow and repeat this wash step

Resuspend pellet in 12 ml Suspension Buffer/RNase
II. **Kit contents**

- Suspension Buffer (150 ml) for suspension of bacterial cell pellets
- RNase A (15 mg) for dissolution in Suspension Buffer
- Lysis Buffer (150 ml) for bacterial cell lysis
- Neutralization Buffer (150 ml) to form a stable cellular debris precipitate
- Equilibration Buffer (70 ml) for equilibrating the columns prior to use
- Wash Buffer (370 ml) for removal of residual impurities
- Elution buffer (200 ml) for plasmid elution
- NucleoBond AX 500 Columns (10 columns) for the isolation step
- Folded filters (10 filters) to eliminate a centrifugation step and to remove cellular debris
- Sealing rings (5 rings) to station the columns in test tubes

III. **Additional materials needed**

- Centrifuge and tubes for harvesting bacterial cultures, capable of $\geq 15,000 \times g$
- Isopropanol
- 70% ethanol
- TE buffer or other low salt buffer
- Tube for collecting and precipitating eluted plasmid DNA
- Funnel for clearing of lysates by folded filters
IV. Protocol for high copy number plasmid DNA

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
<th>Time / x g / Temperature</th>
</tr>
</thead>
</table>
| 1    | Centrifuge bacterial cells from 30 – 150 ml E. coli culture grown in LB medium.  
Discard the supernatant.  
Carefully resuspend the pellet in 12 ml Suspension Buffer + RNase and mix well. | 5 – 10 min / 3000 – 5000 x g / +2 to +8°C |
| 2    | Add 12 ml Lysis Buffer to the suspension and mix gently by inverting the tube 6 to 8 times and incubate.  
*Do not vortex in order to avoid shearing and release of genomic DNA.  
*Do not incubate for more than 5 min to prevent the release of chromosomal DNA from the cell debris.* | 2 – 3 min at +15 to +25°C |
| 3    | Add 12 ml chilled Neutralization Buffer to the suspension.  
Immediately mix the suspension gently by inverting the tube 6 to 8 times until a homogenous suspension is formed.  
*The solution becomes cloudy and flocculent precipitate will form.* | 5 min on ice |
| 4    | Clear the lysate by either centrifugation (4a) or by filtration (4b).  
Centrifuge at high speed  
Directly after centrifugation carefully remove the supernatant from the white precipitate and proceed with step 5. | >45 min / >12,000 x g / +2 to +8°C |
| 4a   | Put a folded filter into a funnel inserted in a 50 ml plastic tube.  
Moisten the filter with a few drops of Equilibration Buffer or PCR grade water.  
The SDS is removed with the Neutralization Buffer (white precipitate) and should not be loaded onto the column. If the supernatant is not clear, load it again onto a folded filter to prevent clogging of the column. | |
| 4b   | Mount the sealing ring on the column as shown in Figure 44 to fix the column in the Collection Tube.  
Insert one column into one Collection Tube.  
Equilibrate the column with 6 ml Equilibration Buffer.  
Allow the column to empty by gravity flow.  
Discard the flowthrough.  
Load the cleared lysate of step 4 onto the equilibrated column.  
Allow the column to empty by gravity flow.  
Discard the flowthrough.  
Wash the column with 16 ml Wash Buffer.  
Allow the column to empty by gravity flow.  
Discard the flowthrough.  
Repeat step 7.  
Discard flowthrough and Collection Tube. |
IV. Protocol for high copy number plasmid DNA, continued

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
<th>Time / x g / Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re-insert the column into a new Collection Tube capable of withstanding high speed centrifugation (≥15,000 x g).</td>
<td>Elute the plasmid with 15 ml Elution Buffer.</td>
<td>Store kit at +15 to +25°C at all times upon arrival.</td>
</tr>
<tr>
<td>Allow the column to empty by gravity flow.</td>
<td>The collected flowthrough contains the plasmid.</td>
<td></td>
</tr>
<tr>
<td>Precipitate the eluted plasmid DNA with 11 ml isopropanol equilibrated to +15 to +25°C.</td>
<td>Centrifuge immediately at high speed.</td>
<td>Carefully discard the supernatant.</td>
</tr>
<tr>
<td>Carefully discard the supernatant.</td>
<td>30 min / ≥15,000 x g / +2 to +8°C</td>
<td></td>
</tr>
<tr>
<td>Wash the plasmid DNA with 4 ml chilled 70% ethanol.</td>
<td>Centrifuge at high speed.</td>
<td>Carefully remove ethanol from the tube with pipette tip.</td>
</tr>
<tr>
<td>Carefully remove ethanol from the tube with pipette tip.</td>
<td>10 min / &gt;15,000 x g / +2 to +8°C</td>
<td>Air-dry the plasmid DNA pellet.</td>
</tr>
<tr>
<td>Carefully re-dissolve the plasmid DNA pellet in 100 – 500 µl TE-buffer or PCR grade water.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

V. Troubleshooting the Genopure Plasmid Maxi protocol

If you get... | Then, the cause may be... | And you should... |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Low nucleic acid yield or purity</td>
<td>Kit stored under non-optimal conditions</td>
<td>Store kit at +15 to +25°C at all times upon arrival.</td>
</tr>
<tr>
<td>Buffers or other reagents were exposed to conditions that reduced their effectiveness</td>
<td>Store all buffers at +15 to +25°C.</td>
<td>After reconstitution of RNase with Suspension Buffer store aliquots at +2 to +8°C.</td>
</tr>
<tr>
<td></td>
<td>Close all reagent bottles tightly after each use to preserve pH, stability and freedom from contamination.</td>
<td></td>
</tr>
<tr>
<td>Reagents and samples not completely mixed</td>
<td>Always mix the sample tube well after addition of each reagent.</td>
<td>Ensure Lysis Buffer and Neutralization Buffer are free of precipitates.</td>
</tr>
<tr>
<td>Low recovery of nucleic acids after elution</td>
<td>Non-optimal reagent has been used for elution. Salt is required for optimal elution</td>
<td>Use the Elution Buffer of the kit.</td>
</tr>
</tbody>
</table>
V. Troubleshooting the Genopure Plasmid Maxi protocol, continued

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low plasmid yield</td>
<td>Too few cells in starting material</td>
<td>Grow <em>E. coli</em> to an absorbency ($A_{600}$) of 2 – 6 before harvest.</td>
</tr>
<tr>
<td>Incomplete cell lysis</td>
<td></td>
<td>Ensure the <em>E. coli</em> pellet is completely resuspended in Suspension Buffer. Ensure the lysate is clear and viscous after the lysis step (incubation with Lysis Buffer). Make sure a cloudy white precipitate forms when Binding Buffer is added to the lysate.</td>
</tr>
<tr>
<td>Lysate did not bind completely to column</td>
<td>Pre-equilibrate the column by adding Equilibration Buffer before adding sample.</td>
<td></td>
</tr>
<tr>
<td>RNA is present in final product</td>
<td>RNase not completely dissolved</td>
<td>To reconstitute the lyophilized RNase completely: 1. Pipette 1 ml of Suspension Buffer into the glass vial containing lyophilized RNase. 2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved. 3. Transfer the reconstituted RNase back into the Suspension Buffer and mix thoroughly. 4. Mark the reconstitute mixture (enzyme and buffer) with the date of reconstitution and store at +2 to +8°C. Reconstituted mixture is stable for 6 months when stored properly.</td>
</tr>
<tr>
<td>Genomic DNA present in final product</td>
<td>Genomic DNA sheared during lysis step.</td>
<td>Vortexing the preparation after addition of Lysis Buffer should be avoided.</td>
</tr>
<tr>
<td>RNase present in final product</td>
<td>RNase not completely dissolved.</td>
<td>See suggestions under “RNA present in final product” above.</td>
</tr>
<tr>
<td>Too many cells in starting material.</td>
<td></td>
<td>Do not overload the column.</td>
</tr>
<tr>
<td>Additional band running slightly faster than supercoiled plasmid is seen on gels</td>
<td>Denatured plasmid in final product.</td>
<td>Reduce the incubation time during step 2 (lysis step) of the protocol.</td>
</tr>
</tbody>
</table>
Typical results with the kit

Figure 45: 1% 1xTAE gel with pUC clones purified with Genopure Plasmid Kit from different *E. coli* strains (500 – 100 ng each). This gel shows that independent from *E. coli* strain high-quality plasmid DNA can be isolated with the kit. Even from strains with higher endonuclease levels no smear, RNA, genomic DNA or linear plasmid DNA is detectable. DNA is suitable for all types of subsequent analysis.

Lanes 1 and 2: JM110
Lanes 3 and 4: Top 10F’ (Invitrogen)
Lanes 5 and 6: BL21
Lanes 7 and 8: DH5α

For further results see page 142

References


Genopure Buffer Set for Low-Copy Number Plasmids

for isolation of low-copy number plasmid DNA in combination with the Genopure Plasmid Kits

Cat. No. 04 634 772 001 (1 set buffer for 20 maxi preps or 60 midi preps)

<table>
<thead>
<tr>
<th>Principle</th>
<th>The Buffer Set is a supplement to the Genopure Plasmid Kits to improve the alkaline lysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting material</td>
<td>The set provides enough additional buffer for:</td>
</tr>
<tr>
<td></td>
<td>20 Maxi preps with the Genopure Plasmid Maxi Kit (each for isolation of up to 500 μg plasmid DNA from 100 to 500 ml bacterial culture), or</td>
</tr>
<tr>
<td></td>
<td>60 Midi preps with the Genopure Plasmid Midi Kit (each for isolation of up to 100 μg plasmid DNA from 10 to 100 ml bacterial culture).</td>
</tr>
<tr>
<td>Application</td>
<td>This set provides extra buffer that may be used with either of the Genopure Plasmid Kits. Doubling the volume of the Suspension, Lysis and Neutralization buffers will help ensure an efficient yield of low copy number plasmids from bacterial culture. The increased buffer volume is required for efficient alkaline lysis.</td>
</tr>
<tr>
<td>Time required</td>
<td>Total time 60 to 75 min (combined use with the Genopure Mini or Maxi Kit) including a filtration step after alkaline lysis. Minimal hands-on time required (about 10 minutes)</td>
</tr>
<tr>
<td>Benefits</td>
<td>Convenient, ready-to-use, function-tested, nuclease-free buffers.</td>
</tr>
<tr>
<td></td>
<td>Helps the Genopure Plasmid Midi and Maxi Kits enhance plasmid yield.</td>
</tr>
<tr>
<td></td>
<td>Ensures reproducible results.</td>
</tr>
</tbody>
</table>
Solution-based Isolation

Overview of Solution-based Isolation 152
DNA Isolation Kit for Mammalian Blood 154
DNA Isolation Kit for Cells and Tissues 162
TriPure Isolation Reagent 170
Overview of Solution-based Isolation

This chapter describes three specialized products. Each features a proprietary cell lysis method that is quicker and safer than standard lysis methods.

All of these products use methods that:

- Can be completed in minutes or hours, instead of days
- Can process multiple samples simultaneously
- Require less handling of potentially hazardous samples
- Minimize the use of toxic organic solvents

For a quick overview of each of these products, continue reading this article. Or, for detailed information on the product most relevant to your research, turn to the page that describes the product in detail:

<table>
<thead>
<tr>
<th>If you are interested in</th>
<th>For preparing</th>
<th>See page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Isolation Kit for Mammalian Blood</td>
<td>Genomic DNA from larger volumes (1 – 10 ml) of mammalian whole blood</td>
<td>154</td>
</tr>
<tr>
<td>DNA Isolation Kit for Cells and Tissues</td>
<td>Genomic DNA from tissues, cultured cells, bacteria, yeast and mouse tail</td>
<td>162</td>
</tr>
<tr>
<td>TriPure Isolation Reagent</td>
<td>Total RNA, DNA, and protein (simultaneously) from cultured cells, blood, tissue, plants, yeast, and bacteria</td>
<td>170</td>
</tr>
</tbody>
</table>

DNA Isolation Kit for Mammalian Blood

Successful genomic Southern hybridizations and long-template PCR start with high quality purified DNA. Traditionally, preparing such DNA from blood required removal of hemoglobin by labor intensive methods such as density gradients and removal of protein and lipids with hazardous solvents such as phenol and chloroform (Sambrook et al., 1989).

The DNA Isolation Kit for Mammalian Blood provides an alternate approach for the isolation of DNA from 1 – 10 ml mammalian whole blood, buffy coat, or lymphocytes. The kit uses a special lysis reagent to selectively lyse erythrocytes. After all erythrocyte components are removed, leukocyte DNA can be isolated free of interfering hemoglobin.

The kit procedure requires approx. 90 min, and can easily process multiple samples. No organic extractions or column purifications are required, yet the isolated DNA is free of both protein and RNA.

The isolated DNA can be used in any application requiring genomic DNA, including long-template PCR and genomic Southern hybridizations.
**DNA Isolation Kit for Cells and Tissues**

The kit offers a convenient, rapid method for the large scale isolation of intact, high molecular weight, genomic DNA from tissues, cultured cells, *E. coli*, yeast, and mouse tails.

The amount of genomic DNA recovered with the DNA Isolation Kit for Cells and Tissues is significantly higher than that obtained using alternative column-based methods in significantly less time. Starting material quantities ranging from 100 mg to 1 gram of tissue, or from 1 x 10^7 to 5 x 10^7 cultured cells, can be analyzed with the kit. Within 2 h plus resuspension time (for tissue samples), the kit yields pure genomic DNA suitable for amplification of long fragments by standard PCR, restriction enzyme digestion, and Southern blotting.

**TriPure Isolation Reagent**

Analysis of gene expression requires clean, intact RNA templates. Isolation of intact RNA is complicated primarily by a host of stable cellular ribonucleases. To minimize RNase activity, RNA isolation procedures typically begin with cell or tissue lysis in a strongly denaturing environment. The RNA is then separated from other cellular components via multiple phenol/chloroform extractions or time-consuming CsCl step gradients.

A simpler alternative, the TriPure Isolation Reagent, offers a rapid RNA isolation procedure that can easily process multiple samples and produces 30 – 150% more RNA than other purification methods. Briefly, the reagent causes RNA, DNA, and protein separate into different organic phases, from which each can be purified by a series of alcohol precipitations (Chomczynski, 1993).

Thus, the TriPure Isolation Reagent allows the simultaneous isolation of RNA, DNA, and protein from the same sample. The isolated total RNA is suitable for Northern blots, RT-PCR, poly(A^+) fractionation, *in vitro* translation, or RNase protection assays. The isolated RNA-free DNA may be used for PCR, restriction digest, or Southern blots. The denatured protein may be analyzed on a Western blot.

**References**


DNA Isolation Kit for Mammalian Blood

for isolation of genomic DNA from 25 samples (10 ml each)

Cat. No. 11 667 327 001

**Principle**
The kit procedure starts with preferential lysis of erythrocytes. The remaining leukocytes are lysed with a strong anionic detergent, and proteins are removed by dehydration and precipitation. The purified genomic DNA is then recovered via ethanol precipitation.

**Starting material**
- 1 – 10 ml whole blood (research samples) that has been treated with an anticoagulant (sodium heparin, sodium citrate, or EDTA)
  - The kit works best with fresh blood or blood stored (at +2 to +8°C or –15 to –25°C) for ≤3 days. It should not be used with blood that is more than 1 month old or blood that has been frozen and thawed more than 3 times.
- Lymphocyte preparations from 10 ml mammalian blood (research samples)
- Buffy coat preparations from 10 – 20 ml mammalian blood (research samples)

**Application**
- For preparation of high molecular weight, purified genomic DNA, which may be used in standard and long-template PCR, Southern blots, etc.
  - The kit has been used to prepare DNA from human, mouse, rat, dog, porcine, guinea pig, monkey, rabbit, and bovine blood.

**Time required**
- Total time: approx. 90 min (plus 30 – 60 min to resuspend DNA)
- Hands-on time: <50 min

**Results**
- Yield: Variable, depending on species (See the table under Part IV of “How to use the kit” in this article).
- Purity: Average $A_{260}/A_{280}$ of isolated DNA = 1.7 – 1.9.

**Benefits**
- **Saves time**, because the kit can prepare DNA directly from whole blood without prior isolation of leukocytes or buffy coat.
- **Increases lab efficiency**, because the kit can prepare multiple DNA samples, free of RNA and protein, in approx. 90 min.
- **Increases lab safety**, because the kit does not require extensive handling of potentially hazardous samples, nor use hazardous organic reagents.
- **Accommodates “real world” samples with varying amounts of leukocytes**, because the sample volume can be adjusted from 1 – 10 ml.
How to use the kit

I. Flow diagram

II. Kit contents
- Red Blood Cell Lysis Buffer (750) ml
- White Blood Cell Lysis Buffer (125) ml
- Protein Precipitation Solution (65 ml)

III. Additional materials needed
- Absolute ethanol, at +15 to +25°C
- 70% ethanol, cold
- Tris-EDTA (TE) buffer, pH 8.0 (or other suitable DNA storage buffer)
- Sterile centrifuge tubes that can hold at least 40 ml (preferably 50 ml) and withstand a centrifugation force of 900 x g
- Sterile 17 x 100 mm tubes that can withstand a centrifugation force of 12,000 x g
- RNase (for optional digestion step)
IV. Expected DNA yield from different mammalian whole blood sources

During testing of this kit, we obtained the following yield data when starting with samples of whole blood that were ≤3 days old:

<table>
<thead>
<tr>
<th>Species</th>
<th>Average yield (μg/10 ml blood)</th>
<th>Yield range (μg/10 ml blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>450</td>
<td>350 – 600</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>160</td>
<td>55 – 295</td>
</tr>
<tr>
<td>Human</td>
<td>350</td>
<td>200 – 700</td>
</tr>
<tr>
<td>Mouse</td>
<td>570</td>
<td>430 – 670</td>
</tr>
<tr>
<td>Pig</td>
<td>670</td>
<td>520 – 780</td>
</tr>
<tr>
<td>Rat</td>
<td>580</td>
<td>350 – 680</td>
</tr>
</tbody>
</table>

![The average A<sub>260</sub>/A<sub>280</sub> ratio of the isolated DNA was 1.7 – 1.9.](image)

Starting with blood that had been stored for 7 days at +2 to +8°C or ≤1 month at –15 to –25°C will lead to a 10 – 15% reduction in yields.

V. Protocol for preparing DNA from 10 ml samples of whole blood

General notes:

- To prepare DNA from buffy coat, lymphocytes, or blood volumes <10 ml, modify this protocol as detailed in the package insert supplied with the kit.
- Before you start preparing the DNA, be sure to:
  - Warm all solutions from the kit to +15 to +25°C.
  - Warm blood samples to +15 to +25°C.

1. For each sample, add 30 ml Red Blood Cell Lysis Buffer to a sterile 50 ml centrifuge tube.

2. To each tube:
   - Add 10 ml mammalian whole blood.
   - Cap the tube and gently mix by inversion.
   - Do not vortex tubes.

3. Mix the tubes by:
   - EITHER placing the centrifuge tube on a rocking platform or gyratory shaker for 10 min.
   - OR inverting the sample at regular intervals by hand, for a total of 10 min.
After the incubation:

- Centrifuge the tube for 10 min at 875 x g.
  - Do not centrifuge the tube at more than 875 x g.
- Look at the supernatant. Is it clear and red?
  - If yes, red cells have lysed completely. Go to Step 5.
  - If no, red cells did not lyse. Stop the protocol; see Part VI below.

4 Carefully pour off and discard the clear, red supernatant.
  - Leave some supernatant with the white cell pellet.

5 Vortex the tube to thoroughly resuspend the white cell pellet in the residual supernatant.

6 For each sample:
   - Add 5 ml White Cell Lysis Buffer, then cap the tube.
   - Mix the contents of the tube thoroughly by vortexing.
     - After vortexing, the solution should be dark brown or red, and contain no undissolved particles.
   - Did the starting sample contain heparin?
     - If no, skip this step and go directly to Step 8.
     - If yes, heat the contents of the tube to +65°C for 10 min to ensure complete white cell lysis.

7 Do you need the final DNA preparation to be free of RNA?
   - If no, skip this step and go directly to Step 9.
   - If yes, remove all RNA from the DNA preparation as follows:
     - To the tube containing DNA, add 0.02 μg RNase per μl DNA solution.
     - Mix the tube gently by inversion.
     - Incubate the tube at +37°C for 15 min.

8 Transfer the sample to a sterile 17 x 100 mm centrifuge tube.

9 For each sample:
   - Add 2.6 ml Protein Precipitation Solution to the tube.
   - Vortex the tube thoroughly (for approximately 25 s).

10 Centrifuge the tube for 10 min at 12,000 x g.

11 Carefully pour the supernatant, which contains the DNA, into a new sterile 50 ml centrifuge tube, then:
   - Add 2 volumes of absolute ethanol +15 to +25°C.
   - Cap the tube.
   - Gently mix the contents of the tube by inversion until the DNA strands precipitate and the remaining liquid is no longer cloudy.

12 Collect the visible strands of DNA by either centrifugation or by spooling:
   - If you want to collect the DNA by centrifugation, then:
     - Centrifuge the tube for 10 min at 875 x g.
     - Discard the supernatant.
     - Add 3 ml of cold 70% ethanol to DNA pellet and resuspend by inversion.
       - Do not vortex.
     - Go to Step 14.
If you want to collect the DNA by spooling, then:

- Wind the DNA strands around a sterile, blunt-ended glass rod.
- Dip the glass rod containing the DNA into 3 ml cold 70% ethanol in a new sterile 50 ml centrifuge tube.
- Swirl the glass rod until the DNA strands are released into the 70% ethanol.
- Go to Step 14.

Centrifuge the tube containing 70% ethanol and DNA for 5 min at 875 x g. Discard the supernatant.

Dry the DNA pellet by:

- EITHER placing the tube under vacuum without heat until the ethanol is no longer visible (usually <5 min).
- OR inverting the tube and allowing the sample to air dry.

To rehydrate and dissolve the DNA pellet:

- Add 1 ml of Tris-EDTA (pH 8.0) (other buffer) to the tube.
- Vortex thoroughly.
- Incubate at +65°C for 30 – 60 min.

For DNA from human blood, use a 30 min incubation. For DNA from other mammalian species, use a 60 min incubation.

- During the incubation, periodically vortex the tubes to facilitate solubilization of the DNA.

Do not incubate the tube for >60 min at +65°C.

Store DNA at +2 to +8°C.

VI. Troubleshooting the DNA Isolation protocol

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
</table>
| A cloudy upper layer and a red lower layer at Step 4 | Little or no red cell lysis | Start with fresh samples and repeat Steps 1 – 4 of the protocol with one or more changes:  
Be sure blood is at +15 to +25°C before starting protocol.  
Use a 15 min incubation in Step 3.  
If mixing by hand in Step 3, invert the tube more frequently. |
| Particles present in sample after vortexing (Step 7) | Incomplete white cell lysis | Using fresh samples, repeat Protocol Va, with one or more changes:  
In Step 6, be sure the white cell pellet is fully resuspended in the residual supernatant after vortexing.  
After adding buffer in Step 7, vortex the sample until the solution is dark brown/red and contains no particles.  
In Step 7, add enough White Blood Cell Lysis Buffer to ensure the solution is not viscous and does not contain clumps of cells.  
In Step 7, incubate the tube at +37°C for 15 – 30 min to facilitate lysis. |
## VI. Troubleshooting the DNA Isolation protocol, continued

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
</table>
| Little or no protein pellet after Step 10 | Ineffective protein precipitation | Using fresh samples, repeat Protocol Va, with one or more changes:  
  ▶ In step 10 vortex the tube for approximately 25 s after adding Protein Precipitation Solution.  
  ▶ Centrifuge the tube at 12,000 x g for at least 10 min. |
| DNA that is difficult to dissolve | Precipitated DNA was overdried in Step 15 | In Step 16, let the DNA rehydrate and dissolve overnight at +2° to +8°C. |
| Low DNA yields | Too few leukocytes in sample | Repeat Protocol Va, with one or more changes:  
  ▶ Use a larger volume of blood (up to 10 ml) as the starting sample.  
  ▶ In Step 7, use less lysis buffer. |
| DNA which does not work in downstream applications | Protein contamination present in DNA \( (A_{260}/A_{280} < 1.6) \) | Follow troubleshooting recommendations under "Little or no protein pellet after Step 10" above. |
| | RNA contamination present in DNA \( (A_{260}/A_{280} > 2.0) \) | Repeat RNase treatment (Step 8), then reprecipitate DNA.  
  ▶ Increase the RNase incubation time to 30 min. |
| | Concentration of DNA not optimal for application | Determine the amount of DNA in the preparation, then use the same amount of DNA in the application that you would normally use if you prepared DNA by another purification method. |
Typical results with the kit

Experiment 1

<table>
<thead>
<tr>
<th>Human Blood</th>
<th>Mouse Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
</tbody>
</table>

Figure 46: Use of DNA from human and mouse blood (prepared with the DNA Isolation Kit for Mammalian Blood) as templates for the amplification of large DNA fragments. Human and mouse blood research samples were collected as previously described (Noeth and Dasovich-Moody, 1997). DNA was prepared from 10 ml of each sample according to the protocol. Aliquots of each DNA preparation were used as templates for the long-template PCR amplification of several gene fragments. Each amplification procedure was performed with the Expand Long Template PCR System, using instructions given in the package insert.

Left panel shows gene fragments amplified from human DNA:
- Lanes 1, 10: DNA Molecular Weight Marker III
- Lanes 2, 3: tPA fragment (9.3 kb) amplified from 25 ng DNA
- Lanes 4, 5: tPA fragment (15 kb) amplified from 50 ng DNA
- Lanes 6, 7: β-globin fragment (23 kb) amplified from 100 ng DNA
- Lanes 8, 9: β-globin fragment (28 kb) amplified from 200 ng DNA

Right panel shows the following fragments amplified from mouse DNA:
- Lanes 1, 10: DNA Molecular Weight Marker III
- Lanes 2, 3: IL-2 gene (4.2 kb) amplified from 330 ng DNA
- Lanes 4, 5: α-2 collagen fragment (5.6 kb) amplified from 100 ng DNA
- Lanes 6, 7: α-2 collagen fragment (10.4 kb) amplified from 50 ng DNA
- Lanes 8, 9: α-2 collagen fragment (15.4 kb) amplified from 100 ng DNA

Result: All fragments were clearly visible on gels.

Experiment 2

<table>
<thead>
<tr>
<th></th>
<th>1 2 3 4 5 6 7 8 9 10 11 12</th>
</tr>
</thead>
</table>

Figure 47: Use of DNA from various human blood research samples (prepared with the DNA Isolation Kit for Mammalian Blood) for detection of the n-ras gene by Southern hybridization. The above protocol was used to prepare DNA from several human blood samples, each of which had been prepared with a different anticoagulant. DNA was also prepared from a lymphocyte preparation and a Buffy coat preparation, using a modification of the protocol outlined in the kit package insert. Ten μg of each preparation was digested with Eco RL electrophoretically separated, and blotted to a nylon membrane. DNA on the membrane was hybridized to a DIG-labeled n-ras probe, and the results visualized chemiluminescently. The samples used were:
- Lanes 1, 12: DNA Molecular Weight Marker VII
- Lanes 2, 3: whole blood, sodium citrate anticoagulant
- Lanes 4, 5: whole blood, heparin anticoagulant
- Lanes 6, 7: whole blood, sodium EDTA anticoagulant
- Lanes 8, 9: Buffy coat preparation
- Lanes 10, 11: Lymphocyte preparation

Result: Each lane contained only a single hybridization band of the expected (7.2 kb) size (Taparowsky et al., 1983).
References

Xiao J. et al. (2010) *Neurology*, **74**: 229 - 238
DNA Isolation Kit for Cells and Tissues

for 10 isolations of 400 mg each for tissue or 5 x 10^7 cultured cells
Cat. No. 11 814 770 001

**Principle**
The DNA Isolation Kit for Cells and Tissues permits the rapid, large-scale isolation of DNA from cells and tissues. This procedure provides a quick, easy, and safe method for removing contaminating RNA and proteins, resulting in purified genomic DNA ranging in size from 50 – 150 kb.

The procedure includes sample homogenization followed by cellular lysis, in the presence of a strong anionic detergent and Proteinase K. RNA is eliminated with an RNase treatment and proteins are removed by selective precipitation and centrifugation. The purified DNA is subsequently recovered by isopropanol precipitation.

**Starting material**
- 100 mg – 1 g tissue (research samples)
- 1 x 10^7 to 5 x 10^7 cultured cells (research samples)
- Up to 10^{11} gram negative bacteria
- Up to 3 x 10^7 yeast cells
- 50 – 400 mg mouse tails

**Application**
- Isolated DNA is suitable for many applications, including standard PCR, long template PCR, and Southern blots.
- DNA isolated can effectively be used with either Taq DNA Polymerase or Expand PCR System products. After quantification we recommend usage of the same amount of DNA per application as typically used in an alternative purification method.

**Time required**
- Hands on time: 35 min
- Total time for tissue: 2.5 h (plus resuspension time)
- Total time for cultured cells:
  - adherent cells/scraped ≥3.5 h (plus resuspension time)
  - adherent cells/trypsinized ≥4.5 h (plus resuspension time)
  - suspension cells ≥4.0 h (plus resuspension time)

**Results**
- Yields are determined via spectrophotometry or fluorometry. The A_{260/280} ratio for isolated DNA samples is typically 1.7 – 1.9.
- For detailed data for cells, tissue, bacteria, yeast or mouse tail please refer to the package insert.

**Benefits**
- **Safe**, eliminates the need for organic extractions or chaotropic reagents.
- **Isolates DNA quickly**, entire procedure can be completed in less than 2.5 hours for tissue (plus resuspension time).
- **Increased DNA recovery**, yield of genomic DNA 2 – 3 times greater than when using column based methods.
- **All reagents necessary** are contained in the kit.
- **Flexible**, can be used with a variety of starting material and varying scales.
How to use the kit

I. Flow diagram

1. Homogenize sample in Cellular Lysis Buffer
2. Add Proteinase K, mix and incubate 1 h at +37°C
3. Add RNase, mix and incubate 15 min at +37°C
4. Add Protein Precipitation Solution, mix and place on ice for 5 min
5. Centrifuge at 26,900 x g for 20 min at +15 to +25°C
6. Transfer supernatant with DNA to a new centrifuge tube
7. Add isopropanol to the supernatant and mix by inversion
8. Centrifuge at 1370 x g for 10 min to pellet the DNA
9. Wash pellet with 70% ethanol
10. Centrifuge at 1370 x g for 5 min
11. Dry DNA pellet shortly and resuspend DNA in TE buffer
12. Purified DNA
II. Kit contents

- Cellular Lysis Buffer, (150 ml)
- Proteinase K Solution (100 μl)
- RNase Solution (5 ml)
- Protein Precipitation Solution (60 ml)

III. Additional materials needed

- 70 % Ethanol
- Isopropanol
- PBS, 1x
- TE Buffer, 1x, pH 8.0 (optional)
- Trypsin (optional)

IV. Procedure for the isolation of DNA from cultured cells

Starting material

- For all procedures, use fresh starting material (tissue, cells from cell culture, bacteria, or yeast) whenever possible.
- If frozen or refrigerated starting material is used, yields may be reduced.

<table>
<thead>
<tr>
<th>Sample material</th>
<th>General information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td>Store solid tissue at −70°C to avoid degradation of the DNA.</td>
</tr>
<tr>
<td></td>
<td>Standard procedure is for a 400 mg sample size. When using less than or greater than 400 mg, follow the alternative procedure, „Optional procedure for DNA isolation from 100 mg – 1 g tissue“.</td>
</tr>
<tr>
<td>Cultured cells</td>
<td>Procedure is written for the isolation of DNA from 5 x 10⁷ cells. Follow the alternative procedure, „Optional procedure for quantities less than 5 x 10⁷ cells“ where appropriate.</td>
</tr>
<tr>
<td>Gram Negative Bacteria</td>
<td>Use up to 10¹¹ cells and follow the procedure titled, „Isolation of DNA from Gram Negative Bacteria or Yeast“.</td>
</tr>
<tr>
<td>(e.g., E. coli)</td>
<td></td>
</tr>
<tr>
<td>Yeast</td>
<td>Count cells and use up to 3 x 10⁶ cells. Follow the procedure titled, „Isolation of DNA from Gram Negative Bacteria or Yeast“.</td>
</tr>
<tr>
<td>Mouse tail</td>
<td>Use 50 – 400 mg mouse tail and follow the procedure titled, „Isolation of DNA from Mouse Tails“</td>
</tr>
</tbody>
</table>

### IVa. Procedure for isolation of DNA from cultured cells

For other sample types see pack insert

**Before you begin**

- Remove the Cellular Lysis Buffer and the Protein Precipitation Solution from +2 to +8°C prior to starting the procedure.

  - **Prior to use,** Resuspend the Cellular Lysis Buffer by placing it at +37°C for approximately 5 min.

- Adjust the waterbaths (+65°C, +37°C) and the centrifuge (+24°C) to the proper temperatures prior to starting the procedure.

Prior to the isolation, count cells via a Coulter Counter or hemocytometer. The following protocols are optimized for 5 x 10⁷ cells. If using fewer cells refer to point IVb.

  - **Pretreatment and harvesting of adherent cells or suspension cell cultures are given in the pack insert.**

**Lysis and RNA removal**

The following procedure applies to 5 x 10⁷ cells (adherent or suspension)

1. Homogenize sample until cells are a fine suspension (approximately 10 – 15 s on a medium setting of a Brinkman Polytron Homogenizer or equivalent).

2. Add 10 μl Proteinase K Solution.

3. Vortex sample for 2 – 3 s to ensure Proteinase K Solution is mixed with the suspension.

4. Place sample at +65°C for 2 hours.

5. Remove sample from +65°C, loosen cap to vent.

6. Add 500 μl RNase Solution to each sample.

   - **Stock concentration of RNase Solution is 10 mg/ml.**

7. Vortex sample for 2 – 3 s to ensure RNase Solution is mixed with the suspension.

8. Place sample at +37°C for 15 min.

   - **The incubation step can be extended up to 1 hour at +37°C if needed.**

**Protein precipitation**

9. Add 6 ml Protein Precipitation Solution to each sample. Vortex thoroughly 5 – 10 s.

   - **Vortexing is necessary for effective removal of protein from the sample.**

10. Place sample on ice for 5 min.

   - **This step is important because it aids in precipitation of the protein.**

11. Centrifuge the sample at 26,900 x g at +15 to +25°C (e.g., 15,000 rpm in a Sorvall RC5B or RC5C).

   - **Ensure that the tube can withstand a centrifugation of 26,900 x g (rmax). If not, transfer sample to another tube at this step (e.g., Nalgene Oak Ridge Centrifuge Tube).**

Samples must be centrifuged at 26,900 x g for a minimum of 20 min. Lower centrifuge temperatures or speeds will result in very loose protein pellets, resulting in reduced yields and contamination of the DNA sample with protein.
### How to use the kit

1. Carefully pipette the supernatant containing the DNA into a new, sterile 50 ml centrifuge tube.  
   Keep the pipette away from the protein pellet!  
   *Pipetting is necessary to avoid white flocculent material at the top of some samples. Pipette from the opposite side of the tube, away from the protein pellet, to ensure that none of the pellet is pipetted into the sample.*

#### DNA precipitation

12. Add 0.7 volumes of isopropanol to the sample.  
13. Invert gently until the upper and lower phases mix.  
   *Usually DNA “strings” will be visible.*  
14. Centrifuge the sample at 1370 x g for 10 min (e.g., 2500 rpm in a Sorvall RT6000B or RT7 centrifuge).  
   Discard the supernatant.  
   *Optional Method: Instead of centrifugation, a sterile blunt-ended glass rod may be used to carefully remove the DNA strands from the isopropanol before transferring them to a new sterile tube containing cold 70% ethanol. Swirl until DNA strands are released into the 70% ethanol. Proceed to step 17.*
15. Save the pellet and add 10 ml cold 70% ethanol to the DNA pellet. Dislodge the pellet from the bottom of the tube by tapping the tube.  
   *This will allow the entire pellet to be washed with the 70% ethanol.*  
16. Centrifuge the sample at 1370 x g for 5 min (e.g., 2500 rpm in a Sorvall RT6000B or RT7 centrifuge).  
17. Discard the supernatant and dry the DNA pellet by placing the sample under vacuum without heat for a few minutes, or until the ethanol is no longer visible.  
   or  
   Allow the sample to air dry.  
   *Do not over-dry the DNA pellet as this will make it much more difficult to fully resuspend the DNA.*  
18. To resuspend the DNA pellet, add 1 ml TE Buffer, pH 8.0, or desired buffer. Tap tube to dislodge the pellet. Place samples at +50°C for 2 hours to aid resuspension or allow to resuspend at +2 to +8°C overnight.  
   *If resulting DNA pellet is quite large, more buffer will be needed to resuspend the sample (e.g., 2 – 3 ml).*  
19. Store samples at +2 to +8°C until ready to use.  
   *If desired, samples can be accurately quantified using spectrophotometry or fluorometry.*
IVb. Optional procedure for isolation of DNA from < 5 x 10^7 cultured cells

**Modifications**

- With slight adjustments to the previous procedure, cell samples from 1 x 10^7 up to 4 x 10^7 can be processed.
- Follow the procedure with the following modification

<table>
<thead>
<tr>
<th>Cell Number</th>
<th>Cellular Lysis Buffer (ml)</th>
<th>Proteinase K Solution (µl)</th>
<th>RNase Solution (µl)</th>
<th>Protein Precipitation Solution (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 10^7</td>
<td>3</td>
<td>2</td>
<td>100</td>
<td>1.2</td>
</tr>
<tr>
<td>2 x 10^7</td>
<td>6</td>
<td>4</td>
<td>200</td>
<td>2.5</td>
</tr>
<tr>
<td>3 x 10^7</td>
<td>9</td>
<td>6</td>
<td>300</td>
<td>3.7</td>
</tr>
<tr>
<td>4 x 10^7</td>
<td>12</td>
<td>8</td>
<td>400</td>
<td>5</td>
</tr>
</tbody>
</table>

**Example:**
For 1 x 10^7 cells, 3.1 ml (volume of Cellular Lysis Buffer + RNase Solution) x 0.4 = 1.2 ml Protein Precipitation Solution

**Expected DNA yield from different cell lines/cell types**

**Average yields for adherent and suspension cells**

Refer to the following table for average DNA yields from various cell lines.

<table>
<thead>
<tr>
<th>Cell line/cell type</th>
<th>Average yield per 5 x 10^7 cells</th>
<th>Range per 5 x 10^7 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOK1/Adherent</td>
<td>977</td>
<td>588 – 1522</td>
</tr>
<tr>
<td>COS1/Adherent</td>
<td>2994</td>
<td>1984 – 4182</td>
</tr>
<tr>
<td>K562/Suspended</td>
<td>684</td>
<td>463 – 885</td>
</tr>
<tr>
<td>PDN-Mouse Hybridoma/Suspended</td>
<td>1298</td>
<td>1220 – 1487</td>
</tr>
</tbody>
</table>
V. Troubleshooting the Cells and Tissues DNA Isolation protocol

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein pellet does not form, pellet is soft, or pellet slides from side of tube.</td>
<td>Centrifuge not at +15 to +25°C</td>
<td>▶ Check temperature of centrifuge. Place at +15 to +25°C prior to beginning the procedure.</td>
</tr>
<tr>
<td></td>
<td>Centrifugation speed too low</td>
<td>▶ Samples must be spun at 26,000 x g $(r_{max})$ for 20 min to form protein pellet.</td>
</tr>
<tr>
<td></td>
<td>Incorrect amount of precipitation buffer used</td>
<td>▶ Always calculate the amount of buffer needed for each precipitation.</td>
</tr>
<tr>
<td></td>
<td>Sample not mixed</td>
<td>▶ Mix sample thoroughly by vortexing 10 s.</td>
</tr>
<tr>
<td></td>
<td>Failure to place on ice</td>
<td>▶ Place on ice 5 min prior to centrifugation to aid in precipitation.</td>
</tr>
<tr>
<td></td>
<td>Incubation of sample following addition of Proteinase K Solution exceeded recommended times</td>
<td>▶ Do not exceed recommended times for incubation as this may result in ineffective protein removal from the DNA sample.</td>
</tr>
<tr>
<td>DNA does not precipitate</td>
<td>Incorrect amount of isopropanol.</td>
<td>▶ Carefully calculate the amount of isopropanol.</td>
</tr>
<tr>
<td></td>
<td>Sample not mixed completely.</td>
<td>▶ Carefully mix sample by inversion until phases disappear.</td>
</tr>
<tr>
<td>Discolored DNA</td>
<td>Certain tissues such as liver may discolor the DNA if the sample is not processed quickly between the isopropanol precipitation and the 70% ethanol wash.</td>
<td>▶ Wash sample with 70% ethanol soon after isopropanol precipitation centrifugation step.</td>
</tr>
<tr>
<td>DNA yield lower than expected</td>
<td>Incomplete lysis</td>
<td>▶ Lysis time and buffer volume not correct for sample size.</td>
</tr>
<tr>
<td></td>
<td>Some samples (i.e., muscle, brain, heart) may have low DNA yields due to difficulty in processing the starting material.</td>
<td>▶ Homogenize muscular tissues until completely in suspension.</td>
</tr>
<tr>
<td>260/280 ratio too high (&gt;1.9)</td>
<td>RNA contamination</td>
<td>▶ RNase treatment insufficient, increase time up to 1 h.</td>
</tr>
<tr>
<td></td>
<td>Sheared DNA</td>
<td>▶ Do not vortex sample unless stated in procedure.</td>
</tr>
</tbody>
</table>
V. Troubleshooting the Cells and Tissues DNA Isolation protocol, continued

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
</table>
| 260/280 ratio too low (<1.7) | Protein contamination | ▶ Increase lysis time and/or amount of Cellular Lysis Buffer.  
▶ Do not exceed recommended incubation times as this may result in ineffective protein removal from the DNA sample.  
▶ Use pipette for sample removal to prevent protein contamination from the pellet. |
| DNA not completely in solution. | | ▶ Heat DNA to +65°C for 30 min to aid in resuspension. |

Typical result with the kit

Figure 48: Amplification of 268 bp to 23 kb genomic DNA fragments isolated with the DNA Isolation Kit for Cells and Tissues. Taq DNA Polymerase, Expand High Fidelity PCR System, and Expand Long Template PCR System were used to amplify fragments isolated with the DNA Isolation Kit for Cells and Tissues.

Lanes 2, 3: Human DMD fragment (268 bp) and mouse c-myc fragment (580 bp) amplified using Taq DNA Polymerase.

Lanes 4, 5, 7 and 8: Human c-myc fragment (1.2 kb), mouse β2 microglobulin fragment (3.6 kb), bovine lysozyme gene fragment (6.9 kb), and human tPA gene fragment (8.3 kb) amplified using Expand High Fidelity PCR System.

Lanes 6, 9 and 10: Mouse α-2 collagen gene fragment (5.6 kb and 10.4) and human β-globin fragment (23 kb) amplified using Expand Long Template PCR System.

Lanes 1 and 11: Molecular Weight Markers VI and II.

References


TriPure Isolation Reagent

Clear, red solution; ready-to-use
Cat. No. 11 667 157 001 (50 ml)
Cat. No. 11 667 165 001 (250 ml)

Principle
During a one-step sample homogenization/lysis procedure, the TriPure Isolation Reagent disrupts cells and denatures endogenous nucleases. After chloroform is added to the extract, the mixture is centrifuged and separates into three phases: a colorless aqueous (upper) phase, a white interphase and a red organic (lower) phase. The phases may then be separated and alcohol precipitation used to recover RNA (from the colorless aqueous phase), DNA and protein (from the interphase and red organic phase).

Starting material
- Cultured cells (research samples)
- Fresh or frozen animal tissue (research samples)
- Human leukocytes (research samples)
- Bacterial cell suspensions
- Yeast spheroplasts
- Plant spheroplasts

Application
- Preparation of total RNA, genomic DNA, and protein from a single biological sample
- DNA-free total RNA may be used for Northern blots, in vitro translation, RNase protection assays, cDNA synthesis, or RT-PCR
- RNA-free DNA may be used for PCR, restriction analysis, Southern blots, and cloning
- Denatured protein may be used for SDS-PAGE and Western blots

Time required
- Total time: approx. 2.5 h for RNA isolation
- Hands-on time: approx. 25 min for RNA isolation

Results
- Yields vary depending on starting material (See the table under Part IV of “How to use the reagent” in this article).
- $A_{260}/A_{280}$ of RNA = 1.6 – 2.0
- $A_{260}/A_{280}$ of DNA >1.7

Benefits
- Saves time, because the RNA isolation procedure requires only 1 h.
- Easy to use, because the red dye in the reagent simplifies identification of different phases.
- Adapts easily to needs of specific laboratories, because the reagent can be used with a wide variety of starting samples.
- Simplifies isolation protocols, because a single reagent can be used to isolate DNA-free RNA, RNA-free DNA, and protein for a variety of applications (see above).
- Increases yield of intact RNA, because the reagent provides an immediate chaotropic denaturing environment that eliminates endogenous RNase activity.
How to use the reagent

I. Flow diagram

(see page 173)

II. Reagent contents

TriPure Isolation Reagent is a clear, red, monophasic solution of phenol and guanidine thiocyanate, pH 4. It is ready to use as supplied.

III. Additional materials needed

For the extraction and phase separation protocol
- Sterile, disposable polypropylene tubes that can withstand 12,000 x g in the presence of TriPure Isolation Reagent and chloroform
- Chloroform (free of all additives such as isoamyl alcohol)

For RNA isolation
- Isopropanol
- 75% ethanol
- Diethylpyrocarbonate (DEPC)-treated, RNase-free water or DEPC-treated 0.5% SDS

For DNA isolation
- Absolute ethanol
- 75 % ethanol
- 8 mM NaOH
- 0.1 M sodium citrate in 10 % ethanol

For protein isolation
- Isopropanol
- 1 % sodium dodecyl sulfate (SDS)
- 0.3 M guanidine hydrochloride (GuHCl) in 95% ethanol
- Absolute ethanol
For particular samples

- Homogenization apparatus (for tissue and certain cells only)
- Red Blood Cell Lysis Buffer, Cat. No. 11 814 389 001 (for white blood cells only)
- Glycogen (for processing <10 mg tissue)

IV. Average nucleic acid yield from various sources

<table>
<thead>
<tr>
<th>Sample</th>
<th>RNA yield</th>
<th>DNA yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tissue:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>6 – 10 μg/mg tissue</td>
<td>3 – 4 μg/mg tissue</td>
</tr>
<tr>
<td>Spleen</td>
<td>6 – 10 μg/mg tissue</td>
<td>not determined</td>
</tr>
<tr>
<td>Kidney</td>
<td>3 – 4 μg/mg tissue</td>
<td>3 – 4 μg/mg tissue</td>
</tr>
<tr>
<td>Skeletal muscle or brain</td>
<td>1.0 – 1.5 μg/mg tissue</td>
<td>2 – 3 μg/mg tissue</td>
</tr>
<tr>
<td>Placenta</td>
<td>1 – 4 μg/mg tissue</td>
<td>2 – 3 μg/mg tissue</td>
</tr>
<tr>
<td><strong>Cultured cells:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>8 – 15 μg/10⁶ cells</td>
<td>not determined</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>5 – 7 μg/10⁶ cells</td>
<td>not determined</td>
</tr>
<tr>
<td>Human, mouse, or rat cells</td>
<td>not determined</td>
<td>5 – 7 μg/10⁶ cells</td>
</tr>
</tbody>
</table>
V. **Protocol for preparing RNA, DNA, and protein from animal tissue** (based on the method of Chomczynski, 1993)

For a detailed, step-by-step procedure and for tips on handling different types of sample, see the package insert supplied with the reagent.

### Extraction

- Add 1 ml TriPure Reagent per 50 – 100 mg tissue
- Homogenize sample in tissue homogenizer
- Incubate 5 min at +15 to +25°C to dissociate nucleoprotein complexes
- Add chloroform (0.2 ml per 1 ml TriPure Reagent)
  - Shake vigorously 15 s; Incubate 2 – 15 min at +15 to +25°C
- Centrifuge at 12,000 x g, 15 min at +2 to +8°C

### 3 Phases:
- Aqueous (containing RNA) colorless
- Interphase (containing DNA) white
- Organic (containing protein) red

### Isolation of RNA

- Transfer aqueous phase to new tube
- Precipitate with isopropanol
  - (0.5 ml per 1 ml TriPure Reagent)
  - Mix by inversion
  - Incubate 5 – 10 min at +15 to +25°C
- Centrifuge at 12,000 x g, 10 min at +2 to +8°C
- Discard supernatant
- Wash pellet 1 x with 75% EtOH (1 ml EtOH per 1 ml TriPure Reagent)
- Centrifuge at 7500 x g, 5 min at +2 to +8°C
- Discard supernatant

### Isolation of DNA/protein

- From remaining interphase and organic phase
- Precipitate with ethanol (EtOH)
  - (0.3 ml 100% EtOH per 1 ml TriPure Reagent)
  - Mix by inversion
  - Incubate 2 – 3 min at +15 to +25°C
- Centrifuge at 2000 x g, 5 min at +2 to +8°C
- Separate pellet and phenol/EtOH supernatant

### Isolation of DNA

- Pellet
- Isolation of protein

### Isolation of protein

- Phenol/EtOH supernatant
- Precipitate with isopropanol
  - (1.5 ml per 1 ml TriPure Reagent)
  - Mix by inversion
  - Incubate 10 min at +15 to +25°C

---

**Solution-based Isolation**

173
TriPure Isolation Reagent

How to use the reagent

1. Air dry pellet
   Resuspend in RNase-free water or DEPC-treated 0.5% SDS
   Incubate 10 – 15 min at +55 to +60°C to resuspend

2. Centrifuge at 2000 x g, 5 min at +2 to +8°C
   Discard supernatant


4. Repeat wash step 2 x

5. Wash pellet in 75% EtOH (1.5 – 2.0 ml per 1 ml TriPure Reagent)
   Incubate 10 – 20 min at +15 to +25°C with occasional mixing

6. Centrifuge at 2000 x g, 5 min at +2 to +8°C
   Discard supernatant

7. Repeat wash step 2 x

8. Briefly dry pellet
   5 – 10 min under vacuum (or air dry)
   Dissolve pellet in 8 mM NaOH

9. Adjust pH to 7.0 – 8.0 with HEPES Buffer and adjust to 1 mM EDTA

10. Store DNA at +2 to +8°C

11. Centrifuge at 12,000 x g, 10 min at +2 to +8°C
    Discard supernatant

12. Resuspend pellet in 0.3 M GuHCl in 95% EtOH
    (2 ml per 1 ml TriPure Reagent)
    Incubate pellet in wash 20 min at +15 to +25°C

13. Centrifuge at 7500 x g, 5 min at +2 to +8°C
    Discard supernatant

14. Repeat wash step 2 x

15. Wash protein pellet in 2 ml 100% EtOH
    Vortex
    Incubate 20 min at +15 to +25°C

16. Centrifuge at 7500 x g, 5 min at +2 to +8°C
    Discard supernatant

17. Dry pellet under vacuum 5 – 10 min (or air dry)
    Add 1% SDS and incubate at +50°C to dissolve pellet
    Sediment insoluble material 10,000 x g, 10 min at +2 to +8°C

18. Transfer supernatant to new tube
    Store protein at –15 to –25°C

19. Store DNA at +2 to +8°C
### V. Troubleshooting the TriPure Isolation protocol

<table>
<thead>
<tr>
<th>During</th>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA isolation</td>
<td>Low RNA yield</td>
<td>Incomplete homogenization or lysis of samples</td>
<td>▶ Use homogenizer to maximize sample yields.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Incomplete solubilization of the final RNA pellet</td>
<td>▶ Do not let RNA pellet dry completely, as a dry pellet will be much less soluble. ▶ Increase incubation time to 30 min at +55°C to solubilize RNA.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insufficient TriPure Reagent used for sample homogenization</td>
<td>▶ Add a sufficient volume of TriPure Isolation Reagent, according to package insert instructions.</td>
</tr>
<tr>
<td></td>
<td>A$<em>{260}$/A$</em>{280}$ ratio &lt;1.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After homogenization, samples were not stored for 5 min at +15 to +25°C</td>
<td>▶ Store at +15 to +25°C for 5 min.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contamination of aqueous phase with phenol phase</td>
<td>▶ Carefully remove the upper aqueous phase for subsequent RNA isolation, making sure to avoid the interphase/organic phase.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Incomplete solubilization of the final RNA pellet</td>
<td>▶ Increase incubation time to 30 min at +55°C to solubilize RNA.</td>
</tr>
<tr>
<td>RNA degradation</td>
<td></td>
<td>Tissues were not immediately processed or frozen after removal from animal</td>
<td>▶ Use fresh tissue or tissue that has been directly frozen in liquid nitrogen and stored at –70°C prior to RNA isolation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Samples used for isolation procedure were stored at –20°C instead of –70°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cells were dispersed by trypsin digestion</td>
<td>▶ Add TriPure Isolation Reagent directly to cells attached to culture dish or flask, according to package insert instructions.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aqueous solutions or tubes were not RNase-free</td>
<td>▶ Use sterile disposable plasticware and pipettes/tips reserved for RNA work only. ▶ Take appropriate precautions to ensure RNase-free environment.</td>
</tr>
<tr>
<td>DNA contamination</td>
<td></td>
<td>Insufficient TriPure Reagent used for sample homogenization</td>
<td>▶ Add a sufficient volume of TriPure Isolation Reagent, according to package insert instructions.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Starting samples contained organic solvents (EtOH, DMSO) or strong buffers; or had an alkaline pH</td>
<td>▶ Carefully remove the upper aqueous phase for subsequent RNA isolation, making sure to avoid the interphase/organic phase.</td>
</tr>
</tbody>
</table>
### V. Troubleshooting the TriPure Isolation protocol, continued

<table>
<thead>
<tr>
<th>During</th>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA isolation</td>
<td>Low DNA yield</td>
<td>Incomplete homogenization or lysis of samples</td>
<td>Use power homogenizer to maximize sample yields.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Incomplete solubilization of the final DNA pellet</td>
<td>Do not let DNA pellet dry completely, as a dry pellet will be much less soluble.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$A_{260}/A_{280}$ ratio &lt;1.7</td>
<td>Incorporate an additional sodium citrate/ethanol wash step.</td>
</tr>
<tr>
<td>DNA degradation</td>
<td>Incomplete removal of phenol from the DNA preparation (during ethanol/sodium citrate wash)</td>
<td>Use fresh tissue or tissue that has been directly frozen in liquid nitrogen and stored at $-70^\circ$C prior to DNA isolation.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DNA degradation</td>
<td>Tissues were not immediately processed or frozen after removal from animal</td>
<td>Avoid using power homogenizer. Use hand-held homogenizer to minimize shearing of high molecular weight DNA.</td>
</tr>
<tr>
<td>RNA contamination</td>
<td>Too much aqueous phase remained with the interphase and organic phase</td>
<td>Carefully remove all of the upper aqueous phase prior to isolation of DNA.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inadequate washing of DNA pellet with 10% EtOH/0.1 M sodium citrate</td>
<td>After adding 1 ml sodium citrate/ethanol for each 1 ml TriPure Isolation Reagent (required in the initial homogenization process) incubate the sample, with occasional mixing, for 30 min at +15 to +25°C.</td>
<td></td>
</tr>
<tr>
<td>Protein isolation</td>
<td>Low protein yield</td>
<td>Incomplete homogenization or lysis of samples</td>
<td>Use power homogenizer to maximize sample yields.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Incomplete solubilization of the final protein pellet</td>
<td>Incubate sample at $+50^\circ$C to completely solubilize the protein.</td>
</tr>
<tr>
<td>Protein degradation</td>
<td>Tissues were not immediately processed or frozen after removal from animal</td>
<td>Use fresh tissue or tissue that has been directly frozen in liquid nitrogen and stored at $-70^\circ$C prior to protein isolation.</td>
<td></td>
</tr>
<tr>
<td>Deformed bands in PAGE analysis</td>
<td>Protein pellet not washed sufficiently</td>
<td>Incorporate an additional wash step.</td>
<td></td>
</tr>
</tbody>
</table>
Typical result with the reagent

Figure 49: Northern blot with total RNA isolated by the TriPure Isolation Reagent. Total RNA was isolated (by the TriPure Reagent protocol) from the following research samples: 1.5 x 10^6 cells of a human leukemia cell line, 5.0 x 10^7 human white blood cells, 1.7 x 10^7 buffy coat cells from human blood, and 500 mg rat tissue. The isolated RNA samples were separated electrophoretically on a gel, transferred to a nylon membrane, and hybridized with a 1 kb, digoxigenin-labeled glyceraldehyde 3-phosphate dehydrogenase (G3PDH) probe. The blot was incubated overnight with DIG System reagents for chemiluminescent detection, then exposed to X-ray film for 5 min. The G3PDH probe recognizes a 1.35 kb mRNA, as shown in:

Lane 1: RNA ladder  
Lanes 2 – 4: RNA from human leukemia cell line  
Lanes 5 – 7: RNA from human white blood cell pellet  
Lanes 8 – 10: RNA from human blood buffy coat  
Lanes 11 – 13: RNA from rat liver tissue

The amount of total RNA applied to the original gel was either 5 μg (lanes 2, 5, 8, 11), 1 μg (lanes 3, 6, 9, 12), or 0.25 μg (lanes 4, 7, 10, 13).

Result: This data clearly demonstrates that high quality, intact RNA is successfully isolated from a variety of starting materials using the TriPure Isolation Reagent.

References

Arslan F. et al. (2010) Circulation, 121, 80 – 90  
Schummer, B. et al. (1998) Technical Tip Biochemica 2, 31 – 33  
Affinity Purification

Overview of Affinity Purification 180
mRNA Capture Kit 184
mRNA Isolation Kit 189
RNA/DNA Stabilization Reagent for Blood/Bone Marrow 197
mRNA Isolation Kit for Blood/Bone Marrow 198
Overview of Affinity Purification

Affinity purification is a versatile and highly specific technique for the purification of all classes of biomolecules utilizing differences in biological activities of chemical structures. The high selectivity of this technique results in good purification and high recovery. Often a concentrating effect is reached which enables large volumes to be conveniently processed.

This section describes products which are based on hybridization properties of certain nucleic acids.

The mRNA kits rely on base pairing between poly (A⁺) residues at the 3’end of mRNAs and the oligo (dT) residues of a biotin-labeled oligo (dT) probe. The biotinylated dT-A hybrid is bound to streptavidin-coated surfaces of either tubes or magnetic particles. Some of the described kits prepare mRNA directly whereas the other one starts with the purification of total nucleic acid and subsequent isolation of the mRNA.

All of these methods:
- Are much faster than traditional nucleic acid isolation methods
- Minimize nucleic acid handling
- Do not require time-consuming centrifugation or electrophoresis steps
- Avoid the use of toxic organic solvents
- Increase the reliability and reproducibility of RNA isolation from human blood and bone marrow research samples
- Are sensitive enough to isolate mRNA from very rare cells circulating in blood (e.g., disseminated tumor cells)

For a quick overview of each of these products, continue reading this article. Or, for detailed information on the product most relevant to your research, turn to the page that describes the product in detail:

<table>
<thead>
<tr>
<th>If you are interested in</th>
<th>For preparing</th>
<th>See page</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA Capture Kit</td>
<td>poly(A⁺) RT-PCR templates from small amounts of total RNA (up to 40 μg), cultured cells (up to 5 x 10⁶), or tissue (up to 20 mg), and the simultaneous immobilization in PCR tubes</td>
<td>184</td>
</tr>
<tr>
<td>mRNA Isolation Kit</td>
<td>poly(A⁺) RNA from larger amounts of total RNA (up to 2.5 mg), cultured cells (up to 10⁸), or tissue (up to 1 g)</td>
<td>189</td>
</tr>
<tr>
<td>RNA/DNA Stabilization Reagent for Blood/Bone Marrow</td>
<td>Stabilized total nucleic acids from 1.5 – 5.0 ml samples of human whole blood or bone marrow aspirates</td>
<td>197</td>
</tr>
<tr>
<td>mRNA Isolation Kit for Blood/Bone Marrow</td>
<td>Highly purified mRNA directly from blood or bone marrow lysates stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow</td>
<td>198</td>
</tr>
</tbody>
</table>
mRNA Capture Kit and mRNA Isolation Kit

The mRNA Capture Kit and the mRNA Isolation Kit are designed to prepare mRNA directly from a variety of starting materials without isolating total RNA. Both can easily process multiple samples simultaneously. Unlike traditional methods, though, the kit procedures take only minutes.

Both kits depend upon the affinity of the poly (A)+ tail of mRNA for a biotin-labeled oligo(dT) probe. The probe can “pull” the mRNA selectively from a lysate without interacting with other RNA or DNA. Once formed, the biotinylated dT-A hybrids can be immobilized on solid surfaces that have been coated with streptavidin, then washed free of unbound contaminants.

In the mRNA Capture Kit, the biotinylated dT-A hybrids are bound to streptavidin-coated PCR tubes, where they can be used directly for RT-PCR. In fact, the entire process, from lysate to final PCR can be done in the same tube. The capture process requires approx. 30 min.

The mRNA Capture Kit contains only reagents for the purification of mRNA; it includes neither reverse transcriptase nor reagents for amplification.

In the mRNA Isolation Kit, the biotinylated dT-A hybrids are bound to streptavidin-coated magnetic particles. The particles are immobilized with a magnetic particle separator. The mRNA is readily released from the particles and is pure enough for all downstream applications, including RT-PCR, Northern blotting, Northern ELISA, and in vitro translation. The isolation process requires approx. 30 min.

mRNA Isolation Kit for Blood/Bone Marrow

The kit relies upon the efficient binding of nucleic acids to silica surfaces in the presence of chaotropic salts and use the silica surface of magnetic glass particles (Figure 50).
Overview of Affinity Purification

Once total nucleic acids are collected on and released from magnetic glass particles, they can be subfractionated. In the kit, poly(A)^+ RNA is isolated from total nucleic acids with biotin-labeled oligo(dT)_20 and streptavidin-coated magnetic particles. The two-step approach (first collect total nucleic acids, then isolate mRNA) produces a highly purified, highly concentrated mRNA preparation.

**Stabilization of total nucleic acids followed by mRNA isolation**

The approach involves two distinct steps: Whole blood samples or bone marrow aspirates (research samples) are lysed and instantaneously stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. Then, mRNA is isolated from the stabilized lysate with the mRNA Isolation Kit for Blood/Bone Marrow. The advantages of this approach are:

- This method involves no cell separation step; thus, there is no danger of losing rare cells that show an aberrant sedimentation or lysis behavior.
- If a sample is mixed with the RNA/DNA Stabilization Reagent immediately after it is drawn, the mRNA in the sample is instantly protected from degradation, even if the stabilized lysate is stored or transported to another location for processing and analysis.

The characteristics of the method are summarized in the following table:

<table>
<thead>
<tr>
<th>Characteristic*</th>
<th>mRNA Isolation Kit for Blood/Bone Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting Material</td>
<td>Blood or bone marrow stabilized with RNA/DNA Stabilization Reagent for Blood/Bone Marrow (up to 5 ml)</td>
</tr>
<tr>
<td>Removal of RBCs</td>
<td>Not required</td>
</tr>
<tr>
<td>Lysis of WBCs</td>
<td>Not required</td>
</tr>
<tr>
<td>Total NA isolation with MGPs</td>
<td>Required</td>
</tr>
<tr>
<td>mRNA isolation with SMPs</td>
<td>Required</td>
</tr>
<tr>
<td>Final mRNA elution volume</td>
<td>20 μl</td>
</tr>
</tbody>
</table>

*Abbreviations: MGPs, magnetic glass particles; NA, nucleic acids; RBCs, red blood cells; SMPs, streptavidin-coated magnetic particles; WBCs, white blood cells

**Advantages of the kit**

- Flexible enough to handle a range of sample sizes
- Sensitive enough to detect rare mRNAs
- Powerful enough to remove inhibitors

We estimate that, to obtain a positive RT-PCR from a research blood sample containing tumor cells (one cell/ml, each with 10 specific mRNA transcripts), you would need to amplify the mRNA from at least 2 ml of whole blood.
Overview of Affinity Purification

Figure 51: Flexibility of the mRNA Kit. The mRNA Isolation Kit for Blood/Bone Marrow was used to isolate mRNA from different volumes of heparinized human blood research samples. An aliquot (30%) of each mRNA was separated electrophoretically, transferred to a membrane by blotting, then analyzed with a DIG-labeled antisense β-actin RNA probe.

Lane A: mRNA from 5 ml blood
Lane B: mRNA from 3 ml blood
Lane C: mRNA from 1.5 ml blood

Result: Each isolated mRNA contained a strong actin band; the amount of the band varied according to the amount of starting material. There was no evidence of degradation products in the preparation.

Figure 52: The effect of rRNA on RT-PCR. Varying amounts of β-actin mRNA (1 ng, panel A; 40 pg, panel B and 1.6 pg, panel C) were mixed with increasing amounts of rRNA, as indicated. The mixtures were used as templates for RT-PCR.

Result: The presence of 2.5 μg rRNA (less than the amount in 1 ml blood) completely inhibited the specific amplification of β-actin mRNA. Yet, in the absence of rRNA, a β-actin amplicon was produced from only 1.6 pg mRNA (equivalent to the mRNA from 1.6 cells).
mRNA Capture Kit

for capturing 192 poly(A)$^+$ RNA preparations in PCR tubes
Cat. No. 11 787 896 001

**Principle**  
A lysis step releases RNA from cultured cells or tissues. The poly(A)$^+$ tail of mRNA in the lysate hybridizes to biotin-labeled oligo(dT). A streptavidin-coated PCR tube immobilizes the biotin-labeled dT-A hybrids and washes remove unbound contaminants. The oligo(dT) part of this hybrid also serves as a primer for reverse transcriptase in RT-PCR. The entire process (including RT-PCR) takes place in a single tube.

*The mRNA Capture Kit contains only reagents for the purification of mRNA; it includes neither reverse transcriptase nor reagents for amplification.*

**Starting material**  
Research samples may contain up to:
- 40 μg total RNA
- 5 x 10$^5$ cultured cells
- 20 mg tissue

**Application**  
Preparation of highly purified poly(A)$^+$ RNA, which may be used directly for qualitative or quantitative RT-PCR

**Time required**  
- Total time: approx. 30 min (starting from total RNA)
- Hands-on time: <25 min (starting from total RNA)

**Results**  
- Yield: Variable, depending upon starting material (See Part IV of “How to use the kit” in this article).
- Purity: When isolated mRNA is used as template for RT-PCR, the amplicons are clearly visible and free of background (See “Typical results with the kit” in this article).

**Benefits**  
- **Significantly reduces the time needed to isolate mRNA**, because the kit uses a semi-automated process that easily handles multiple samples.
- **Minimizes sample loss, handling time, and contamination**, because the kit allows rapid, efficient isolation of mRNA and RT-PCR in a single tube.
- **Improves the reliability and reproducibility of RT-PCR**, because the kit produces excellent RT-PCR templates, even from small amounts of starting material or material containing low-abundance mRNAs.
How to use the kit

I. Flow diagram

II. Kit contents

- Lysis Buffer containing lithium dodecyl sulfate (50 ml)
- Oligo(dT)20 Concentrate: Oligo(dT)20, biotin-labeled, 20x concentrated (50 μl)
  
  *Dilute the Oligo(dT)20 Concentrate just before use.*

- PCR grade water (1 ml)
- Wash Buffer, ready-to-use (500 ml)
- Streptavidin-coated PCR Tubes (192)
- Caps for PCR Tubes (192)
III. Additional materials needed

- Sterile cups (for preparing dilutions)
- Pipettes with sterile, aerosol-resistant pipette tips
- Syringe fitted with a 21-gauge needle (for tissue and cultured cells only)
- PBS (for preparing cultured cells only)
- Mortar, pestle, liquid nitrogen (for preparing tissue samples only)
- PCR thermal cycler
- Reagents for RT-PCR
- Electrophoresis equipment or other equipment for analyzing PCR products

IV. Typical mRNA content of different samples

<table>
<thead>
<tr>
<th>Starting material</th>
<th>Total RNA (μg)</th>
<th>mRNA (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured cells (10^7 cells)</td>
<td>30 – 500</td>
<td>0.3 – 25</td>
</tr>
<tr>
<td>Tissue (100 mg):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse brain</td>
<td>200</td>
<td>7</td>
</tr>
<tr>
<td>Mouse liver</td>
<td>700</td>
<td>14</td>
</tr>
<tr>
<td>Mouse lung</td>
<td>130</td>
<td>10</td>
</tr>
</tbody>
</table>

V. Protocol for capturing mRNA from total RNA, cultured cells, or tissue

General notes:

- Make sure that all material which contacts the RNA is free of contaminating RNases. For decontamination of equipment, see Appendix (page 219).
- For tips on preventing RNase contamination in this procedure, as well as guidelines for the subsequent RT-PCR procedure, see the package insert provided with the mRNA Capture Kit.

1. To prepare the Oligo(dT)_20 Working Solution used in Step 3 below, dilute the Oligo(dT)_20 Concentrate 1:20 with PCR grade water.

   Prepare the Oligo(dT)_20 Working Solution just before use; prepare only as much as you will need. (Each capture requires 4 μl of Working Solution.)

2. Prepare the sample:

   - Total RNA: Dilute ≤40 μg of total RNA in 200 μl of Lysis Buffer.
   - Cultured cells: Wash cells (≤5 x 10^5) twice with ice-cold phosphate-buffered saline (PBS). Add 200 μl of Lysis Buffer to the cell pellet. Shear DNA mechanically by passing the sample 6 x through a 21-gauge needle.
   - Tissue: Snap freeze ≤20 mg of tissue. Grind frozen tissue to a homogeneous powder in a pre-cooled mortar. Chill 200 μl of Lysis Buffer to ≤0°C in a sodium chloride-ice water bath, then add the frozen powder to the chilled Lysis Buffer. Homogenize the powder suspension by passing it 4 x through a 21-gauge needle. Centrifuge the suspension at 11,000 x g for 30 s. Use only the supernatant for the capture procedure.

   If possible, perform all steps in the capture procedure below at a temperature between 0°C and –4°C. Otherwise, perform these steps at +4°C.
Hybridize the mRNA to biotin-labeled oligo(dT)$_{20}$:

- Add 4 μl Oligo(dT)$_{20}$ Working Solution to the sample to form the hybridization mix.
- Incubate the hybridization mix for:
  - EITHER 5 min at +2 to +8°C (if you started with tissue)
  - OR 5 min at +37°C (if you started with cultured cells or total RNA)

Immobilize mRNA in the Streptavidin-coated PCR Tubes:

- Add 50 μl of the hybridization mix to a Streptavidin-coated PCR tube.
  - Use 4 tubes to process entire hybridization mix.
- Incubate the hybridization mix for:
  - EITHER 3 min at +2 to +8°C (if you started with tissue)
  - OR 3 min at +37°C (if you started with cultured cells or total RNA)

Wash the immobilized sample 3 times. For each wash:

- Add 250 μl Wash Buffer to the tube.
  - To avoid losing mRNA, do not wash the sample too vigorously.
- Remove all Wash Buffer and discard.
  - After the third wash, the captured mRNA in the Streptavidin-coated PCR Tube is ready for RT-PCR.

**VI. Troubleshooting the mRNA Capture protocol**

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>No PCR product (after RT-PCR)</td>
<td>Insufficient homogenization of sample material</td>
<td>Eliminate all visible clumps in lysate (Step 1) by homogenization, etc.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mRNA trapped inside sample clumps can not be isolated by this procedure.</td>
</tr>
<tr>
<td>Too much starting material per volume of Lysis Buffer (lysate viscosity too high)</td>
<td>Do not use more than the recommended amount of starting material.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dilute the lysate (Step 1) before continuing the procedure.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The viscosity of the lysate is critical to successful mRNA isolation.</td>
</tr>
<tr>
<td>Degradation of RNA by contaminating RNase from buffers or equipment</td>
<td>Check all buffers for RNase contamination.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Follow procedures in the package insert for RNase decontamination.</td>
</tr>
<tr>
<td>Degradation of RNA because RNases in sample material were not sufficiently inactivated</td>
<td>Follow the kit procedure exactly as written and be especially careful to:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Work rapidly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perform indicated steps at ice water temperatures (0 to –4°C)</td>
</tr>
</tbody>
</table>
**Typical result with the kit**

**Figure 53: Detection of MCAD and β-actin transcripts in K562 cells.** Lysates from 10⁶ cells were serially diluted. Messenger RNA from the dilutions was captured in Streptavidin-coated PCR tubes according to the above protocol. RT-PCR was performed in the same PCR tubes with reagents from the Titan One Tube PCR System and primers derived from the human MCAD (Medium-chain acyl-CoA dehydrogenase) and β-actin genes. Amplicons were run on a 1% agarose gel. For details of the procedure and the primers used, see Zoelch and Frey (1996).

- **Lanes 1, 10, 18:** DNA Molecular Weight Marker VI
- The remaining lanes contained mRNA from
  - **Lane 2:** 1 x 10⁴ cells
  - **Lanes 3, 11:** 2 x 10³ cells
  - **Lanes 4, 12:** 400 cells
  - **Lanes 5, 13:** 80 cells
  - **Lanes 6, 14:** 16 cells
  - **Lanes 7, 15:** 3.2 cells
  - **Lanes 8, 16:** 0.64 cells
  - **Lanes 9, 17:** Control (no RT performed)

**Result:** The mRNA Capture Kit and Titan One Tube RT-PCR System allow the efficient detection of β-actin and MCAD template RNA’s from as few as 3.2 K562 cells.

**References**

# mRNA Isolation Kit

for the isolation of poly(A)$^{+}$ RNA (at least 70 μg)

Cat. No. 11 741 985 001

## Principle

The poly(A)$^{+}$ tail of mRNA hybridizes to a biotin-labeled oligo(dT) probe. Streptavidin-coated magnetic particles capture the biotinylated dT-A hybrids. A magnetic particle separator collects the magnetic particles, and washes remove contaminants. Then, water elutes the mRNA from the particles.

## Starting material

Research samples may contain up to:

- 2.5 mg total RNA
- $10^6$ cultured cells
- 1 g tissue

## Application

Preparation of highly purified poly(A)$^{+}$ RNA, which may be used for RT-PCR, cDNA synthesis, Northern blotting, Northern ELISA, RNase protection assays, *in vitro* translation, etc.

## Time required

- **Total time:** approx. 30 min (starting from total RNA)
- **Hands-on time:** approx. 15 min (starting from total RNA)

## Results

- **Yield:** Variable, depending upon starting material (See Part IV of “How to use the kit” in this article).
- **Purity:** Isolated mRNA, free of DNA and other RNAs.

## Benefits

- **Saves time,** because the kit can isolate mRNA directly from cell lysates and tissue homogenates; no isolation of total RNA required.
- **Accommodates a wide range of samples,** and allows both small and large scale preparations of mRNA.
- **Increases lab safety,** because the kit does not use hazardous organic solvents.
I. Flow diagram

- Total RNA
- Culture cell lysate
- Tissue homogenate

Hybridization of mRNA and biotinylated oligo (dT)

Capturing on streptavidin-coated magnetic particle

Magnetic separation

Elution of mRNA
II.  Kit contents

- Lysis Buffer containing lithium dodecyl sulfate (100 ml)
- Streptavidin-coated Magnetic Particles (1.7 ml)
- Oligo(dT)$_{20}$, biotin-labeled, ready-to-use (66 μl)
- Wash Buffer (50 ml)
- PCR grade water (4 ml)
- Storage Buffer (7 ml)

III.  Additional materials needed

- Magnetic Particle Separator
- Sterile tubes and cups
- Pipettes with sterile, disposable pipette tips
- Syringe fitted with a 21-gauge needle (for tissue and cultured cells only)
- PBS (for preparing cultured cells only)
- Mortar, pestle, liquid nitrogen (for preparing tissue samples only)

IV.  Typical mRNA content of different samples

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<thead>
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<td>14</td>
</tr>
<tr>
<td>Mouse lung</td>
<td>130</td>
<td>10</td>
</tr>
</tbody>
</table>

V.  Protocol for isolating mRNA from 500 μg total RNA, 2 x 10^7 cultured cells, or 200 mg tissue

General notes:

- Make sure that all material which contacts the RNA is free of contaminating RNases. For decontamination of equipment, see Appendix (page 219).
- The procedures below apply only to the isolation of mRNA from the specified amounts of starting material (500 μg total RNA, 2 x 10^7 cultured cells, or 200 mg tissue). To isolate mRNA from different amounts or volumes of sample, you must alter the amounts of reagents used in the procedures below. For details, see the package insert provided with the mRNA Isolation Kit.

Prepare the sample:

- Total RNA: Dilute 500 μg total RNA (up to 200 μl) with Lysis Buffer (final total volume, 400 μl). Incubate for 2 min at +65°C.

Do not dilute the Lysis Buffer more than twofold.
Cultured cells: Wash cells \((2 \times 10^7)\) twice with ice-cold phosphate-buffered saline (PBS). Add 3 ml Lysis Buffer to the cell pellet. Shear DNA mechanically by passing the sample 6 x through a 21-gauge needle.

Tissue: Snap freeze 200 mg of tissue. Grind frozen tissue to a homogeneous powder in a pre-cooled mortar. Chill 3 ml Lysis Buffer to \(\leq 0^\circ\text{C}\) in a sodium chloride-ice water bath, then add the frozen powder to the chilled Lysis Buffer. Homogenize the powder suspension by passing it 4 x through a 21-gauge needle. Centrifuge the suspension at 11,000 \(x\ g\) for 30 s. Use only the supernatant for the capture procedure.

Perform all steps in the capture procedure below at a temperature between 0°C and –4°C.

Prepare the Streptavidin-coated Magnetic Particles:
1. Resuspend Streptavidin-coated Magnetic Particles thoroughly and pipette 300 μl Streptavidin-coated Magnetic Particles into a fresh cup or tube.
2. Immobilize the Streptavidin-coated Magnetic Particles on the side of the container with a Magnetic Particle Separator.
3. Remove the storage buffer from the Streptavidin-coated Magnetic Particles.
5. Again immobilize the Streptavidin-coated Magnetic Particles with the magnetic separator and remove all the Lysis Buffer.

Never let the Streptavidin-coated Magnetic Particles dry out.

Hybridize the mRNA to the Biotin-labeled Oligo(dT)_{20}:
1. Add 3 μl Biotin-labeled Oligo(dT)_{20} to the sample (from Step 1)
2. Mix sample and Oligo(dT)_{20} well to form the hybridization mix.

Immobilize the biotinylated dT-A hybrids with Streptavidin-coated Magnetic Particles:
1. Add hybridization mix to the tube containing the prepared Streptavidin-coated Magnetic Particles (from Step 2).
2. Resuspend Streptavidin-coated Magnetic Particles in the hybridization mix.
3. Incubate for:
   - EITHER 5 min at +37°C (if you started with total RNA or cultured cells).
   - OR 5 min at 0°C (if you started with tissue).

Separate the Streptavidin-coated Magnetic Particles from the fluid with a Magnetic Particle Separator.

This step takes about 3 min.

Wash the Streptavidin-coated Magnetic Particles 3 times. For each wash:
1. Add 500 μl Wash Buffer to the tube.
2. Resuspend the Streptavidin-coated Magnetic Particles in the Wash Buffer.
3. Separate the Streptavidin-coated Magnetic Particles from the buffer with a Magnetic Particle Separator.
4. Remove all Wash Buffer and discard.

Elute the mRNA:
1. Add 50 μl redistilled water to the Streptavidin-coated Magnetic Particles.
2. Resuspend the Streptavidin-coated Magnetic Particles in the redistilled water.
3. Incubate for 2 min at +65°C.
4. Separate Streptavidin-coated Magnetic Particles from the eluate with a Magnetic Particle Separator.

Transfer the supernatant (containing the mRNA) to a fresh RNase-free tube.

To quantitate the mRNA spectrophotometrically, assume that 1 \(\text{A}_{260}\) unit corresponds to a concentration of 40 μg/ml.
### VI. Troubleshooting the mRNA Isolation protocol

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low yield or no yield</td>
<td>Insufficient homogenization of sample material</td>
<td>Eliminate all visible clumps in lysate (Step 1) by homogenization, etc.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>mRNA trapped inside sample clumps cannot be isolated by this procedure.</strong></td>
</tr>
<tr>
<td>Too much sample material per volume of Lysis Buffer (lysate viscosity too high)</td>
<td>Use no more than the recommended amount of starting material.</td>
<td><strong>The viscosity of the lysate is critical to successful mRNA isolation.</strong></td>
</tr>
<tr>
<td>Elution temperature too low</td>
<td>Incubate suspension in PCR grade water for 2 min at +65°C (Step 7).</td>
<td></td>
</tr>
<tr>
<td>Elution volume too low</td>
<td>Repeat elution.</td>
<td></td>
</tr>
<tr>
<td>Improper preparation and reuse of magnetic particles</td>
<td>Follow instructions in the package insert if you intend to reuse the Streptavidin-coated Magnetic Particles.</td>
<td><strong>Do not regenerate Streptavidin-coated Magnetic Particles with alkali.</strong></td>
</tr>
<tr>
<td>Wrong lysis buffer or lysis buffer with too much guanidine thiocyanate (GTC)</td>
<td>Use only the Lysis Buffer provided in the kit.</td>
<td>If you use a lysis buffer containing GTC, follow the guidelines in the package insert for GTC concentration and hybridization temperature.</td>
</tr>
<tr>
<td>RNase contamination in buffers</td>
<td>Check all buffers for RNase contamination.</td>
<td>Follow published procedures (e.g., Farrel, 1983) for RNase decontamination.</td>
</tr>
<tr>
<td>Degraded mRNA</td>
<td>RNase contamination in buffers</td>
<td>Check all buffers for RNase contamination.</td>
</tr>
<tr>
<td></td>
<td>Sample material processed too slowly or at a too high temperature</td>
<td>Follow published procedures (e.g., Farrel, 1983) for RNase decontamination.</td>
</tr>
<tr>
<td>rRNA or DNA contamination</td>
<td>Too much sample material per volume of lysis buffer (lysate viscosity too high)</td>
<td>Use no more than the recommended amount of starting material.</td>
</tr>
<tr>
<td></td>
<td>Use the isolated mRNA as starting material and repeat the isolation protocol.</td>
<td></td>
</tr>
</tbody>
</table>
Typical results with the kit

Experiment 1

Figure 54: Northern blot with liver mRNA prepared with the mRNA Isolation Kit. mRNA was isolated from liver tissue according to the above protocol. The mRNA was separated electrophoretically, transferred to a membrane by Northern blotting, and analyzed with a DIG-labeled actin cDNA probe. The hybrids were visualized with an enzyme-labeled anti-DIG antibody and a chemiluminescent enzyme substrate.

Result: A single, strong actin band was visible in the mRNA (left lane). No degradation products were visible.

Experiment 2

Detection of Angiotensin Converting Enzyme (ACE) mRNA in chicken eye.

[submitted by T.H. Wheeler-Schilling, M. Munz, E. Guenther, and K. Kohler; Department of Pathophysiology of Vision and Neuroophthalmology, Division of Experimental Ophthalmology, University Eye Hospital Tuebingen, Roentgenweg 11, D-72076 Tuebingen, Germany]

Background: In the last decade, research has increasingly focused on the role of peptides in the physiology of the central nervous system and in the retina. Specifically, attention focused on the renin-angiotensin system (RAS), in which angiotensinogen is cleaved by renin and angiotensin-converting enzyme (ACE) to produce the active peptide angiotensin II (Oparil and Haber, 1974).

Originally, the RAS was thought to be solely a circulatory endocrine system. However, increasing evidence suggests that an RAS is expressed locally in various tissues. In these tissues, the RAS is believed to act as a functionally independent paracrine/autocrine system (Unger et al., 1991).

By using RT-PCR, we have been able to detect ACE-specific mRNA in several tissues from chicken eye. To obtain enough mRNA template from these neuronal tissues, we used the mRNA Isolation Kit.

Methods: Preparation of mRNA from chicken eye. The eyes of each chicken (Gallus domesticus, 12 days old) were dissected immediately after enucleation by hemisection along the ora serrata. The optic nerve head, pecten, retina, choroid, and the iris/ciliary body were carefully prepared and frozen in liquid nitrogen. Tissues were homogenized and mRNA isolated with the mRNA Isolation Kit, according to the protocol given in the kit package insert.

Reverse transcription. Isolated mRNA samples were treated with RNase-free DNase, extracted with phenol, and precipitated with ethanol, according to standard procedures. The treated mRNA samples were reverse transcribed with the 1st Strand cDNA Synthesis Kit for RT-PCR (AMV).
ACE-specific PCR. Optimal PCR conditions were determined with the PCR Optimization Kit. The entire product (20 μl) of the reverse transcription reaction was amplified in a 100 μl reaction mix containing 10 mM Tris HCl (pH 8.8), 75 mM KCl, 1.5 mM MgCl2, 3% dimethylsulfoxide, 10 mM dNTPs, 15 pmol of each ACE-specific primer, and 2.5 units Taq polymerase. The sequences of the ACE-specific primers were deduced from EMBL; AC: L40175 (Esther et al., 1994):

5’-ACAACAAGACCAATGAGGTGC-3’ (sense primer)

5’-CCTTCCAGGCAAAGAGGAG-3’ (antisense primer)

After an initial denaturation step (5 min, +95°C), samples were subjected to 30 PCR cycles [1 cycle = denaturation (45 s, +94°C), annealing (1 min, +60°C), and elongation (1 min, +72°C)]. The final extension was 10 min at +72°C.

Results and conclusions: We obtained at least 3 μg pure mRNA per chicken retina (i.e., 8.7 – 9.3 μg mRNA/100 mg tissue) in <1 h with the mRNA Isolation Kit. The yield was highly reproducible (data not shown).

Starting from the isolated mRNA, the RT-PCR produced an ACE-specific 525 bp-amplicon (Figure 55). The specificity of the amplified sequence was tested by digestion of the amplicon with Pvu II. As expected, the amplicon was cleaved into two fragments (137 bp and 388 bp) (Figure 55).

These results indicate that ACE gene expression occurs in the retina, choroid, pecten, optic nerve and iris/ciliary body of the chicken eye. The results strongly support the hypothesis that ACE-specific mRNA is localized in retinal neurons or glia.
mRNA Isolation Kit

Typical results with the kit

References


Bönsch C. et al. (2010) *J. Virol.*, **84**: 11737 - 11746


RNA/DNA Stabilization Reagent for Blood/Bone Marrow

for cell lysis and stabilization of nucleic acids contained in blood or bone marrow
Cat. No. 11 934 317 001

**Principle**

The purification of mRNA from blood or bone marrow requires two steps:

1. Cells from fresh blood or bone marrow are lysed with RNA/DNA Stabilization Reagent for Blood/Bone Marrow, which contains guanidine isothiocyanate and detergent. The Stabilization Reagent inhibits nuclease activity to protect nucleic acids in the lysate from degradation.

2. After lysis, total nucleic acids are purified with the mRNA Isolation Kit for Blood/Bone Marrow.

The RNA/DNA Stabilization Reagent for Blood/Bone Marrow, described on this page, is used only for Step 1 of the procedure. The mRNA Isolation Kit for Blood/Bone Marrow, described on the following page, is used only for Step 2 of the procedure.

**Starting material**

- Anticoagulant-treated research samples (1.5 – 5.0 ml) of:
  - Human blood
  - Human bone marrow aspirates

  *The anticoagulant may be EDTA, citrate, or heparin.*

**Application**

- Rapid stabilization of nucleic acids from human whole blood or bone marrow research samples

**Time required**

- Total time: <5 min (for lysis of sample and stabilization of nucleic acids in lysate)
- Hands-on time: <5 min

**Results**

- Nucleic acids stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow may be stored up to 1 year at –15 to –25°C, ≤1 day at +2 to +8°C, or ≤6 h at +20 to +25°C.

**Benefits**

- **Immediately stabilizes** nucleic acids from freshly drawn blood or bone marrow, so that they can be stored for later analysis or transport to a central laboratory.
- **Protects** nucleic acids in the lysed sample from degradation by nucleases.
mRNA Isolation Kit for Blood/Bone Marrow

for isolating mRNA from blood or bone marrow lysates

Cat. No. 11 934 333 001

Principle

The purification of mRNA requires two steps:

1. Cells from fresh blood or bone marrow are lysed with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow.

2. After lysis, total nucleic acids released from blood or bone marrow are bound to Magnetic Glass Particles, washed free of cellular contaminants, then released from the Magnetic Glass Particles. Total nucleic acids are incubated with biotin-labeled oligo(dT) and Streptavidin-coated Magnetic Particles. The mRNA is bound to the Oligo(dT)-Streptavidin-coated Magnetic Particle complex, while other nucleic acids (DNA, rRNA, tRNA) are washed away. Purified mRNA is then released from the Streptavidin-coated Magnetic Particles.

The RNA/DNA Stabilization Reagent for Blood/Bone Marrow, described on the previous page, is used only for Step 1 of the procedure. The mRNA Isolation Kit for Blood/Bone Marrow, described on this page, is used only for Step 2 of the procedure.

Starting material

- Lysates containing nucleic acids from 1.5 – 5.0 ml human blood or bone marrow aspirates (research samples), obtained and stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow

Application

- Isolation of highly purified, human mRNA, which is suitable for RT-PCR, cDNA synthesis, nuclease protection assays, Northern blotting, or Northern ELISA

Time required

- Total time: 1.5 h (for isolation of mRNA from stabilized lysate)
- Hands-on time: approx. 1 h

Results

- Typical mRNA yield: approx. 100 ng/ml of human blood.

Since white blood cell count can differ significantly between donors, mRNA yield can range from 50 to 200 ng/ml blood.

- Variance in yield (aliquots from one donor): ≤10%.

- Sensitivity: In a model system, it was possible to detect melanoma mRNA (by RT-PCR) in a kit-prepared sample containing mRNA from 5 ml of fresh blood that had been spiked with mRNA from 5 melanoma cells (Mel-Ju).

Benefits

- **Isolates nucleic acids** without prior cell separation, ensuring representation of all cells in final mRNA isolate.

- **Increases the efficiency** of RT-PCR screening by removing PCR inhibitors (rRNA, tRNA, and hemoglobin) from the mRNA template.

- **Allows detection** of very rare mRNA species.

- **Produces high yields** of purified, intact mRNA, suitable for multiple research applications.

- **Increases** the reliability and reproducibility of mRNA isolation.
How to use the reagent and the kit

I. Flow diagram

1. Lyse red blood cells and stabilize total nucleic acids with RNA/DNA Stabilization Reagent for Blood/Bone Marrow (separate reagent; not contained in kit)

2. Bind total nucleic acids to Magnetic Glass Particles and collect by centrifugation

3. Wash the Magnetic Glass Particles 4 times and collect them with a Magnetic Particle Separator after each wash

4. Elute total nucleic acids from Magnetic Glass Particles (Eluent may be used for DNA analysis)

5. Immobilize mRNA with biotinylated Oligo(dT) and Streptavidin Magnetic Particles

6. Collect Streptavidin Magnetic Particles with a Magnetic Particle Separator and wash 3 times (as in Step 3)

7. Elute mRNA from Streptavidin-coated Magnetic Particles

RNA/DNA Stabilization Reagent for Blood/Bone Marrow is not contained in the kit
II. Kit contents

- Magnetic Glass Particles (5 x 50 tablets)
- Magnetic Glass Particles Wash Buffer, 5x concentrated (2 x 20 ml)

Add 160 ml absolute ethanol to concentrated buffer to form the working Magnetic Glass Particle Wash Buffer used in Protocol IVb.

- Magnetic Glass Particles Elution Buffer (33 ml)
- Hybridization Buffer (17 ml)
- Oligo(dT)$_{20}$ Probe, biotin-labeled (330 μl)
- Streptavidin-coated Magnetic Particles (3.3 ml, suspension)
- Streptavidin Magnetic Particles Wash Buffer (110 ml)
- PCR grade water (18 ml)

III. Additional materials needed

- RNA/DNA Stabilization Reagent for Blood/Bone Marrow, with guanidine isothiocyanate and Triton X-100 (500 ml)

The reagent crystallizes at temperatures below +20°C. Check solution for the absence of crystals before use, and warm to +37°C if it is not fully dissolved. Thoroughly mix before use.

- Absolute ethanol
- Dry ice (optional, for short-term storage of sample lysates)
- Graduated cylinder or tube (for measuring blood/bone marrow volume)
- 50 ml centrifuge tubes, screw-cap, sterile
- Centrifuge for 50 ml tubes, capable of an 1100 x g centrifugal force
- 2 ml microcentrifuge tubes
- Tabletop microcentrifuge
- Magnetic Particle Separator
- Shaker for 2 ml tubes, for incubation at +37°C and +70°C
- Vortex mixer
- Roller incubator, for incubation at +20 to +25°C
IV. Protocols for preparing mRNA from 5 ml of human whole blood or bone marrow research samples

When handling blood, bone marrow, and blood/bone marrow lysates, take the precautions you usually take when handling potentially hazardous material. Dispose of all supernatants properly.

IVa. Lysis of cells from 5 ml of sample and stabilization of total nucleic acid

Sample preparation: Draw sample (blood or bone marrow) into a tube containing an anticoagulant (EDTA, citrate, or heparin). Label the tube with all relevant information (identification, kind of sample, total volume, date and time of draw, delay between draw and processing).

1. In a screw-cap container (bottle or tube) that will hold at least 55 ml:
   - Pour 50 ml prewarmed and fully dissolved RNA/DNA Stabilization Reagent for Blood/Bone Marrow.
   - Add 5 ml of sample.
   - Mix contents of container vigorously, for instance by vortexing.

2. Do one of the following:
   - If you are going to isolate mRNA immediately, go to Procedure IVb.
   - If you are going to store or transport the sample lysate to a central laboratory before isolating mRNA, go to Step 3.

3. Between now and the start of Procedure IVb, do one of the following:
   - Store lysate as follows:
     - Up to 1 year at −15 to −25°C
     - ≤1 day at +2 to +8°C.
     - ≤6 h at +20 to +25°C.
   - Place the lysate on dry ice for transport to another facility.

IVb. Isolation of mRNA from 55 ml of lysate (equivalent to 5 ml of sample)

For preparation of mRNA from small samples (1.5 – 3 ml blood or bone marrow), see the package insert supplied with the mRNA Isolation Kit for Blood/Bone Marrow.

Sample preparation: All lysates should be at +20 to +25°C. If the sample lysates have been frozen prior to this procedure, thaw them carefully and prewarm to +20 to +25°C. Thoroughly mix (e.g., by vortexing) to ensure crystallized material is fully dissolved.

1. Resuspend 8 Magnetic Glass Particles tablets in 480 μl PCR grade water.

2. In a sterile, screw-cap 50 ml centrifuge tube:
   - Add entire sample lysate from Procedure IVa.
   - Add Magnetic Glass Particles suspension from Step 1 and cap the tube.
   - Vortex for 10 s.
   - Incubate for 30 min at +20 to +25°C on a roller incubator.
After the incubation:
- Centrifuge the tube for 2 min at 1100 x g and +15 to +25°C.
- Discard the supernatant.
- Invert the tube and place on filter paper for 30 s. Discard the supernatant properly, as you would any potentially hazardous material.

Transfer the Magnetic Glass Particles to a 2 ml microcentrifuge tube, then wash them 4 times. For each wash:
- Resuspend Magnetic Glass Particles in 1 ml working Magnetic Glass Particle Wash Buffer with a pipette.
- Collect the Magnetic Glass Particles on the side of the tube with a Magnetic Particle Separator.
- Remove and discard all the supernatant.
- Wait a few seconds, then remove (and discard) any residual supernatant.

To elute the total nucleic acids from the Magnetic Glass Particles:
- Resuspend Magnetic Glass Particles in 1 ml Magnetic Glass Particle Elution Buffer.
- Incubate suspension for 5 min on a 1400 rpm shaker at +70°C.
- Collect the Magnetic Glass Particles on the side of the tube with a Magnetic Particle Separator.
- Immediately transfer the supernatant to a fresh 2 ml tube.
- Again use a Magnetic Particle Separator to remove any residual Magnetic Glass Particles from the supernatant.
- Immediately transfer the supernatant to a fresh 2 ml tube. This supernatant contains the total nucleic acids from the sample.

To isolate mRNA from the total nucleic acids:
- Prepare 100 μl Streptavidin Magnetic Particles: Transfer 100 μl SMP suspension to a fresh 2 ml tube. With a Magnetic Particle Separator, collect the Streptavidin Magnetic Particles from the suspension on the side of the tube. Withdraw and discard the supernatant (storage solution) from the tube.
- Prepare 0.5 ml Hybridization Reagent: Add 10 μl biotin-labeled Oligo(dT)$_{20}$ Probe to 0.5 ml Hybridization Buffer and mix thoroughly.
- Add 0.5 ml Hybridization Reagent to the total nucleic acids supernatant (from Step 5) and mix. Incubate for 2 min at +37°C.
- Transfer the Hybridization Reagent-nucleic acid supernatant mixture to the tube containing 100 μl Streptavidin Magnetic Particles and resuspend the Streptavidin Magnetic Particles with a pipette.
- Let the tube stand for 5 min at +37°C.
- Collect Streptavidin Magnetic Particles on the side of the tube for 3 min with a Magnetic Particle Separator.
- Discard the supernatant.

Wash the Streptavidin Magnetic Particles 3 times. For each wash:
- Resuspend Streptavidin Magnetic Particles in 0.3 ml Streptavidin Magnetic Particle Wash Buffer with a pipette.
- Collect the Streptavidin Magnetic Particles on the side of the tube with a Magnetic Particle Separator.
- Remove and discard all the supernatant.
To elute the mRNA from the Streptavidin Magnetic Particles:

1. Resuspend Streptavidin Magnetic Particles in 20 μl PCR grade water with a pipette.
2. Let the tube stand for 2 min at +70°C.
3. Collect the Streptavidin Magnetic Particles on the side of the tube with a Magnetic Particle Separator.
4. Immediately transfer supernatant to a fresh 2 ml tube.
5. Again use a Magnetic Particle Separator to remove any residual Streptavidin Magnetic Particles from the supernatant.
6. Immediately transfer the supernatant to a fresh 2 ml tube.

The supernatant (from Step 8) contains the mRNA from the sample.

You may:

- EITHER use the isolated mRNA directly in RT-PCR or other applications.
- OR store the isolated mRNA at −15 to −25°C or −80°C.

### V. Troubleshooting the Stabilization and the mRNA Isolation protocol

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low yield of mRNA (&lt;50 ng/ml blood)</td>
<td>RNase contamination in reagents or equipment</td>
<td>See the Appendix, page 219 of this manual, for general guidelines on handling RNA.</td>
</tr>
<tr>
<td>Improper storage of lysate before mRNA isolation</td>
<td>For optimal mRNA stability, store lysates:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 year at −15 to −25°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 day at +2 to +8°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 h at +20 to +25°C</td>
<td></td>
</tr>
<tr>
<td>Improper storage of blood before stabilization</td>
<td>Add RNA/DNA Stabilization Reagent for Blood/Bone Marrow to blood within a few hours after drawing.</td>
<td></td>
</tr>
<tr>
<td>Fragmented mRNA</td>
<td>RNase contamination in reagents or equipment</td>
<td>See the Appendix, page 219 of this manual, for general guidelines on handling RNA.</td>
</tr>
<tr>
<td>Improper storage of lysate before mRNA isolation</td>
<td>For optimal mRNA stability, store lysates:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 year at −15 to −25°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 day at +2 to +8°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 h at +20 to +25°C</td>
<td></td>
</tr>
</tbody>
</table>
Typical results with the kit

Experiment 1

Figure 56: Stability of mRNA after lysis in RNA/DNA Stabilization Reagent for Blood/Bone Marrow.
A total of 6 ml normal human heparinized blood research sample was lysed and stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. The lysate was divided into three aliquots.

Lanes A: One aliquot was stored at +2 to +8°C
Lanes B: One aliquot was stored at +15 to +25°C
Lanes C: One aliquot was stored at –15 to –25°C

After 4 days, duplicate samples were taken from each aliquot and mRNA was isolated from each, according to Protocol IVb. The isolated mRNA was separated electrophoretically, transferred to a membrane by Northern blotting, and analyzed with a DIG-labeled antisense β-actin RNA probe.

Result: The same band was clearly visible in each sample. However, the sample stored at –15 to –25°C (lanes C) contained more of the band than the sample stored at +2 to +8°C (lanes A), which in turn contained more than the sample stored at +15 to +25°C (lanes B).
Experiment 2

Figure 57: Isolation of mRNA from bone marrow and blood (research samples). Normal human heparinized bone marrow and blood were lysed with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. mRNA was isolated from each sample with the mRNA Isolation Kit for Blood/Bone Marrow. The mRNA from 0.8 ml bone marrow (Lane A) and from 1.0 ml of blood (Lane B) was separated electrophoretically, transferred to a membrane by Northern blotting, and analyzed with DIG-labeled antisense β-actin RNA probe.

Result: A single, sharp β-actin band was visible in each sample, which indicates the isolated mRNA was undegraded.

Reference

Gault, J. et al. (2005) Stroke, 36, 872 – 874
LÓPEZ P. et al. (2008), J Rheumatol., 35, 1559 - 1566
de PAZ B. et al. (2010) J Rheumatol, 37: 2502 - 2510
Suárez, A. et al. (2005) Ann Rheum Dis, 64, 1605 – 1610
Gel Filtration

Overview of Gel Filtration 208
Quick Spin Columns 210
mini Quick Spin Columns 214
Overview of Gel Filtration

This chapter describes a set of “spin columns” that:

- Are ready to use
- Take just minutes to purify nucleic acids from unincorporated nucleotides, linkers, or excess primers
- Fit in most common tabletop/clinical centrifuges and low-speed floor model centrifuges (Quick Spin Columns) or
- Fit in microcentrifuges (mini Quick Spin Columns)

For a quick overview of each of these products, continue reading this article. Or, for detailed information on the product most relevant to your research, turn to the page that describes the product in detail:

<table>
<thead>
<tr>
<th>If you are interested in</th>
<th>For preparing</th>
<th>See page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quick Spin Columns</td>
<td>Labeled DNA or RNA, free of nucleotides and other small molecules</td>
<td>210</td>
</tr>
<tr>
<td>mini Quick Spin Columns</td>
<td>Labeled DNA, RNA or oligonucleotides, free of nucleotides and other small molecules</td>
<td>214</td>
</tr>
</tbody>
</table>

**Principle of gel filtration chromatography**

All the products described in this chapter depend on gel filtration chromatography, which separates molecules based on their relative size. This type of chromatography was originally described by Porath and Flodin (1959) for the desalting of proteins.

If a molecule can enter and exit the pores of the gel matrix, its rate of movement is determined by the flow of the chromatographic buffer and the diffusion properties of the molecule. The matrix thus functions like a molecular sieve. Smaller molecules enter and leave many pores of the matrix, thus traversing the length of the column relatively slowly. Larger molecules do not enter the gel pores and therefore elute rapidly from the column.
Overview of the Quick Spin method

Roche Applied Science has adapted gel filtration technology to a spin column format for use in a wide range of applications, including removal of:

- Unincorporated nucleotides from labeling reactions
- Fluorescent dye-labeled dideoxy terminators.

Quick Spin Columns are:

- **Simple**
  Prespin column, apply an undiluted sample to the column bed, and centrifuge the column for a few minutes to recover the purified DNA or RNA.

- **Fast**
  Quick Spin Columns for radiolabeled DNA or RNA purification can separate unincorporated radionucleotides from labeled DNA or RNA in approximately 6 min.

- **Convenient**
  The columns are ready-to-use and are even supplied with collection tubes.

- **Longer than most spin columns**
  Longer columns provide more effective separation, leading to higher yields of DNA or RNA, with fewer contaminating nucleotides, linkers or primers.

- **Quality tested**
  Columns are quality tested according to the strict current quality control procedures to ensure the absence of nucleases, maximum recovery of nucleic acid, and maximum retention of small unincorporated molecules.

Reference

Quick Spin Columns

G-25 or G-50 Sephadex Columns for Radiolabeled DNA Purification
G-25 or G-50 Sephadex Columns for Radiolabeled RNA Purification

Cat. No. 11 273 922 001 20 columns for DNA purification (Sephadex G-25)
Cat. No. 11 273 949 001 50 columns for DNA purification (Sephadex G-25)
Cat. No. 11 273 965 001 20 columns for DNA purification (Sephadex G-50)
Cat. No. 11 273 973 001 50 columns for DNA purification (Sephadex G-50)
Cat. No. 11 273 990 001 20 columns for RNA purification (Sephadex G-25)
Cat. No. 11 274 015 001 20 columns for RNA purification (Sephadex G-50)

Principle
Quick Spin columns contain gel filtration matrices which allow large molecules (e.g., DNA or RNA) to pass through quickly while retaining small molecules (e.g., nucleotides). The Quick Spin format improves the molecular sieving concept by using centrifugation to separate DNA or RNA rapidly and cleanly from small contaminants.

Starting material
- Nucleic acids from labeling reactions

Application
- Quick and efficient removal of non-incorporated precursors from:
  - Labeled DNA (from nick translation, end-labeling, polymerization, and other labeling reactions)
  - Labeled RNA (from end-labeling or polymerization reactions)

Time required
- Total time: 8 min
- Hands-on time: 2 min

Results

**G-50 Sephadex Columns**
- Exclusion limit:
  - DNA: 72 bp / RNA: 72 bp
- Recovery:
  - DNA: ≥90 % / RNA: ≥80 %
- Retention of nucleotides:
  - DNA: >99 % / RNA: >99 %
- Sample volume:
  - DNA: up to 100 μl / RNA: up to 100 μl

**G-25 Sephadex Columns**
- Exclusion limit:
  - DNA: 10 – 12 bp / RNA: 10 – 12 bp
- Recovery:
  - DNA: ≥80 % / RNA: ≥80 %
- Retention of nucleotides:
  - DNA: ≥95 % / RNA: ≥95 %
- Sample volume:
  - DNA: up to 50 μl / RNA: up to 50 μl

Benefits
- **Saves time and effort**, because columns are ready-to-use.
- **Provides more nucleic acids in less time**, because fewer steps mean minimal sample loss and increased yield.
- **Gives reproducible results**, because columns are quality tested for:
  - High recovery of radiolabeled DNA, RNA, and poly(A).
  - Maximum retention of unincorporated nucleotides.
  - Absence of DNases in DNA columns.
  - Absences of DNases and RNases in RNA columns.
How to use the columns

I. Flow diagram

1. Resuspend column matrix by inversion & drain by gravity
2. Spin column (1100 x g, 2 min) in a swinging-bucket rotor (to remove residual buffer)
3. Apply sample to center of column bed (Maximum sample volume: G-50, 100 µl; G-25, 50 µl)
4. Spin column (1100 x g, 4 min) in swinging-bucket rotor
5. Recover eluate containing the DNA/RNA

II. Package contents

- Each column contains 0.8 ml bed volume of pre-swollen Sephadex G-25 (fine) or G-50 (fine) in STE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, 100 mM NaCl).
- Each column unit contains a ready-to-use column plus two collection tubes (one for draining the column buffer and one for collecting the purified nucleic acid sample).

⚠️ The Quick Spin columns for radiolabeled RNA purification are packed in ziplock bags to prevent contamination.

III. Additional materials needed

- Tabletop or low-speed floor model centrifuge
- Swinging-bucket rotor
IV. Protocol for purifying radiolabeled nucleic acids

1. Remove the column from the storage bag, and gently invert it several times to resuspend the matrix.

2. Remove most of the buffer from the column as follows:
   - Remove the top cap from the column, then remove the bottom tip.
   - Drain the column by gravity, then discard the eluate.

3. Remove residual buffer from the column as follows:
   - Place the column in a collection tube.
   - Centrifuge at 1100 x g for 2 min in a swinging-bucket rotor.
   - Discard the collection tube with the eluted buffer.

4. While keeping the column upright, very carefully apply the sample to the center of the column bed.
   - Apply 20 – 50 μl sample to a G-25 column; 20 – 100 μl to a G-50 column.

5. While keeping the column upright, place the column in the second collection tube.

6. Centrifuge the tube at 1100 x g for 4 min in a swinging-bucket rotor.

7. Save the eluate from the second collection tube. It contains the purified sample.

8. Discard the column in a radioactive waste container.

V. Troubleshooting the Quick Spin protocol

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor sample recovery</td>
<td>Tipping of the column, which causes backflow of sample and reduced nucleic acids recovery</td>
<td>Keep the column upright during and after application of sample, especially after centrifugation.</td>
</tr>
<tr>
<td>Wrong g-force used during centrifugation</td>
<td></td>
<td>Use 1100 x g for centrifugation spins.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Be sure the centrifuge is correctly calibrated.</td>
</tr>
<tr>
<td>Wrong rotor</td>
<td></td>
<td>Use swinging-bucket rotor.</td>
</tr>
<tr>
<td>Too much or too little DNA/RNA loaded on column</td>
<td>Apply a sample containing 0.02 – 1.0 mg/ml nucleic acid. At &gt;1.0 mg/ml, the sample is viscous and may not migrate through the columns easily, leading to poor recovery and/or contamination with smaller molecules. At &lt;0.02 mg/ml, DNA/RNA recovery may be low.</td>
<td>To improve recovery of dilute samples, add carrier (glycogen, tRNA or sperm DNA) to the sample.</td>
</tr>
</tbody>
</table>

A fixed-angle rotor will cause lower yields.
### V. Troubleshooting the Quick Spin protocol, continued

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleotide contamination of final sample</td>
<td>Sample applied to side of the column matrix, allowing nucleotides to flow around the matrix</td>
<td>▶ Carefully apply the sample to the center of the column matrix.</td>
</tr>
<tr>
<td>Column overloaded, which causes nucleotides to flow through the matrix</td>
<td></td>
<td>▶ Apply a sample containing 0.02 – 1.0 mg/ml nucleic acid.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>▶ Apply no more than 50 μl sample to a G-25 column; no more than 100 μl to a G-50 column.</td>
</tr>
<tr>
<td>Wrong g-force during centrifugation</td>
<td></td>
<td>▶ Use 1100 x g for centrifugation spins.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>▶ Be sure the centrifuge is correctly calibrated.</td>
</tr>
<tr>
<td>Wrong rotor</td>
<td></td>
<td>▶ Use swinging-bucket rotor.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A fixed-angle rotor may cause nucleotide contamination of final sample.</em></td>
</tr>
</tbody>
</table>

### Reference


mini Quick Spin Columns

Ready-to-use, microcentrifuge-compatible chromatography columns for quick and efficient purification of nucleic acids from labeling reactions

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 814 419 001</td>
<td>50 mini Quick Spin DNA Columns</td>
<td></td>
</tr>
<tr>
<td>11 814 427 001</td>
<td>50 mini Quick Spin RNA Columns</td>
<td></td>
</tr>
<tr>
<td>11 814 397 001</td>
<td>50 mini Quick Spin Oligo Columns</td>
<td></td>
</tr>
</tbody>
</table>

**Principle**

The method uses gel filtration chromatography, which separates molecules based upon their relative size. During centrifugation, mini Quick Spin Columns allow larger molecules (DNA, RNA, or oligonucleotides) to pass through quickly while retaining smaller molecules (such as unincorporated nucleotides). The rapid separation of larger from smaller molecules may be performed in a conventional tabletop microcentrifuge.

**Starting material**

- For ‘DNA or RNA’ columns: 20 – 75 μl nucleic acid labeling mixture
- For ‘Oligo’ columns: 20 – 50 μl oligonucleotide labeling mixture

**Application**

The mini Quick Spin columns are designed for quick and complete removal of unincorporated nucleotides (e.g., radionucleotides or fluorescent dye-labeled dideoxy terminators) from labeled nucleic acids that have been prepared by nick translation, end labeling, polymerization, or other labeling techniques. Specifically:

- Use mini Quick Spin DNA Columns to purify radiolabeled or fluorescent dye-labeled DNA (≥20 bp) with ≥90 % recovery
- Use mini Quick Spin RNA Columns to purify radiolabeled RNA (≥20 bases) with ≥80 % recovery
- Use mini Quick Spin Oligo Columns to purify radiolabeled oligonucleotides (≥8 bases) with ≥80 % recovery

**Time required**

- Hands-on time: 2 min
- Total time: 7 min (additional 1 min if buffer exchange is needed)

**Results**

Removal of unincorporated nucleotides

- mini Quick Spin DNA or RNA Column ≥99 % retention
- mini Quick Spin Oligo Column ≥90 % retention

Recovery of labeled probes

- mini Quick Spin DNA Columns ≥90 %
- mini Quick Spin RNA/Oligo Columns ≥80 %

Exclusion limit

- mini Quick Spin DNA/RNA Columns ≥20 bp
- mini Quick Spin Oligo Columns ≥8 bases

**Benefits**

- Saves time and effort, because columns are ready to use with any standard microcentrifuge.
- Provides more nucleic acid in less time, because two-step procedure means minimal sample loss and increased yield.
- Gives reproducible results, because columns are quality tested to ensure high recovery of labeled DNA, RNA and oligonucleotides with a maximum retention of unincorporated nucleotides.
How to use the columns

I. Flow diagram

The following flow diagram summarizes the steps for preparing any mini Quick Spin Column and purifying a nucleic acid sample with the column.

<table>
<thead>
<tr>
<th>Preparing the column</th>
<th>Purifying the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resuspend column matrix</td>
<td>Carefully apply sample to center of column bed</td>
</tr>
<tr>
<td>Remove top cap, then snap off bottom tip, and place column in sterile microcentrifuge tube</td>
<td>Spin column (1000 x g, 4 min)</td>
</tr>
<tr>
<td>Spin column (1000 x g, 1 min) to pack the column matrix and remove residual buffer</td>
<td>Recover eluate containing the nucleic acids</td>
</tr>
</tbody>
</table>

II. Additional materials needed

- Variable speed microcentrifuge
- 1.5 ml sterile microcentrifuge tubes (2 per column)

III. Preparing the column

Use the following procedure to prepare any mini Quick Spin Column.

1. Evenly resuspend the Sephadex matrix in the column buffer by doing either of the following:
   - Invert the column vigorously several times.
   - Vortex gently for 3 – 5 s at low speed.
   - Do not vortex the column at medium or high speed or for periods longer than 5 s. Excessive vortexing may crush the matrix and lead to contamination on the purified sample with unincorporated nucleotides.

2. To prevent the formation of a vacuum (which can cause uneven buffer flow), remove the ends from the column in the following order:
   - First, remove the top cap from the column.
   - If the cap is filled with Sephadex, put the cap back on the column and remix column contents (as in step 1) until most of the matrix is in the body of the column rather than in the cap.
   - Then, snap off the bottom tip.
mini Quick Spin Columns

How to use the columns

3. Remove excess buffer and pack the column as follows:
   - Place column in a sterile 1.5 ml microcentrifuge tube.
   - Place the tube in a microcentrifuge rotor.
   - To attach the rotor lid properly, turn the microcentrifuge tube so that the flip-top cap faces the inside of the rotor. (There is a V-shaped notch in the support ring of the column to help align the column with the rotor.)
   - Centrifuge at 1000 x g for 1 min at +15 to +25°C.
   - Discard the collection tube with the eluted buffer.
   - During packing, the column matrix normally pulls away from the sides of the tube.

4. Is the isolated nucleic acid to be used in a fluorescent sequencing reaction?
   - If no, then skip this step and go to step 5.
   - If yes, then exchange the buffer in the column for water, as follows:
     - Place the packed column in a 1.5 ml microcentrifuge tube.
     - While keeping the column upright, apply 300 μl PCR grade water to the center of the column bed.
     - Centrifuge the tube at 1000 x g for 2 min at +15 to +25°C.
     - Discard the collection tube and eluted buffer.
     - Go to step 5.
   - The extra buffer exchange step minimizes the amount of salt in the final purified nucleic acid. Minimal salt in the final product means that, when concentrated, the sample will run cleanly in sequencing applications.

5. Use the column immediately (as detailed below).
   - Delay will allow the column matrix to dry out. A dry column will not perform properly.

IV. Purifying the sample

After preparing any mini Quick Spin Column according to the procedure described above, use the procedure below to purify a nucleic acid sample with the prepared column.

6. While keeping the column upright, do the following:
   - Place the prepared column in a clean, sterile 1.5 ml microcentrifuge tube.
   - Very slowly and carefully apply the sample to the center of the column bed. Do not apply the sample to the side of the column. Any sample on the side of the column will bypass the separation matrix and will arrive in the collection tube without being fractionated.
   - Use 20 – 50 μl sample for the mini Quick Spin Oligo Column; 20 – 75 μl sample for the mini Quick Spin DNA or RNA Column. Do not overload the column.

7. Centrifuge the tube at 1000 x g for 4 min at +15 to +25°C in the microcentrifuge.

8. Save the eluate in the second collection tube. It contains the purified nucleic acid.
   - Discard the mini Quick Spin Column in an appropriate waste receptacle.
## V. Troubleshooting the mini Quick Spin protocol

This table describes various problems that may occur during the purification procedures and recommendations for avoiding them.

<table>
<thead>
<tr>
<th>If you get...</th>
<th>Then, the cause may be...</th>
<th>And you should...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution of final sample</td>
<td>Excess packing buffer wasn’t removed before sample application</td>
<td>Before applying sample, centrifuge column at 1000 x g for 1 min to pack the matrix. Discard eluate.</td>
</tr>
<tr>
<td>Purified nucleic acid contaminated with unincorporated nucleotides</td>
<td>Sample applied to sides of column, allowing molecules to flow around, rather than through the matrix (without purification)</td>
<td>Apply sample directly to center of the column bed.</td>
</tr>
<tr>
<td>Column overloaded</td>
<td>Do not apply more than the maximum recommended sample volume.</td>
<td></td>
</tr>
<tr>
<td>Centrifugation speed too fast, causing column matrix to collapse and unincorporated nucleotides to pass freely through column</td>
<td>Do not centrifuge the columns faster than the recommended speed.</td>
<td></td>
</tr>
<tr>
<td>Column was vortexed too long or too vigorously during matrix resuspension</td>
<td>Do not vortex the column for longer than 5 s.</td>
<td></td>
</tr>
<tr>
<td>Poor recovery or no recovery of nucleic acid</td>
<td>Centrifugation speed too fast (see above) or centrifugation time too short</td>
<td>Do not centrifuge the columns faster than the recommended speed.</td>
</tr>
<tr>
<td>Matrix not evenly resuspended prior to packing step</td>
<td>To fully resuspend the matrix before packing step, do one of the following:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Invert column vigorously several times and flick the column sharply to help resuspend the matrix.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vortex column gently (5 s or less, low speed).</td>
<td></td>
</tr>
<tr>
<td>Sample volume too small (&lt;20 μl)</td>
<td>Do one of the following:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Add 1x STE buffer to sample until the total sample volume is 20 μl.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>After applying sample, add 1x STE buffer to the matrix.</td>
<td></td>
</tr>
</tbody>
</table>

*Total volume applied (sample + STE buffer) MUST NOT be greater than the maximum sample volume recommended for the column.*
Typical result with the columns

Figure 58: Removal of Unincorporated Radiolabeled Nucleotides from 5’ End-labeled Oligomers using the Mini Quick Spin DNA and Oligo Columns. Various oligomers, ranging in size from 32 bp to 8 bp, were end-labeled using the 5’ End-Labeling Kit and γ32P-ATP and purified using either the mini Quick Spin DNA or Oligo Columns to remove unincorporated nucleotide, per manufacturer’s instructions. One fifth of the isolated sample was electrophoresed through a 20% acrylamide gel in 1x TBE for 5 – 6 hours at 150 V. The gel was overlaid with plastic wrap and exposed to Lumi-Film for approximately 18 h.

Lane 1: Oligo Marker Control
Lane 2: Oligo Marker/mini Quick Spin DNA Column
Lane 3: Oligo Marker/mini Quick Spin DNA Column
Lane 4: –
Lane 5: Oligo Marker Control
Lane 6: Oligo Marker/mini Quick Spin Oligo Column
Lane 7: Oligo Marker/mini Quick Spin Oligo Column
Lane 8: –
Lane 9: Oligo Marker Control

Result: Removal of Unincorporated Radio-labeled Nucleotides from 5’ End-labeled Oligomers using the Mini Quick Spin DNA and Mini Quick Spin Oligo Columns

References

Automated Nucleic Acid Isolation

MagNA Lyser Instrument 220
MagNA Pure LC Instrument 222
MagNA Pure Compact Instrument 223
Automated Sample Preparation

PCR and real-time PCR application require precise and reproducible nucleic acid purification. For optimized and rapid automated sample preparation, Roche Applied Science offers systems with harmonized workflows:

- **MagNA Lyser Instrument**
  for homogenization of solid sample materials

- **MagNA Pure LC System**
  for nucleic acid purification (32 samples per run) and PCR set-up

- **MagNA Pure Compact System**
  for nucleic acid purification (8 samples per run)

MagNA Lyser Instrument

The MagNA Lyser Instrument is a benchtop device that automatically disrupts cells or other biological materials. The instrument facilitates the production of a supernatant containing nucleic acids and proteins suitable for subsequent purification, extraction, or analysis.

- Simplify labor-intensive sample preparation.
- Efficiently homogenize a wide variety of sample materials.
- Perform consistent and reproducible sample disruption.
- Prevent nucleic acid degradation with the benchtop cooling block.
- Ease your setup and cleanup with a removable rotor and prefilled disposable vials.

- Automate with an easy-to-use instrument.
- Homogenize up to 16 samples in just a few seconds.
- Save valuable lab space with a small benchtop instrument.
- Reduce hands-on time by replacing the mortar and pestle and other manual methods.

- Integrate your workflow with the automated nucleic acid isolation of the MagNA Pure LC System and the MagNA Pure Compact System.
**MagNA Lyser Workflow**

During a MagNA Lyser Instrument run, the rotor, which is filled with special tubes, rapidly oscillates. The oscillation of the instrument agitates the contents of the tubes (i.e. beads, cell material and lysing reagents) up and down at extremely high speed with a slight twisting motion.

The cells in the sample tubes are disrupted nearly instantaneously when they collide with the ceramic and glass beads. The rate of collision and energy of impact (both of which determine the effectiveness of the disruption process) depend on the shaking speed of the instrument and the specific gravity of the beads. By varying both of these parameters, optimal disruption of a wide variety of cells can be ensured. The time of the run can also be varied to disrupt different types of tissue efficiently.

For detailed information, visit [www.magnapure.com](http://www.magnapure.com) or contact your local representative.
MagNA Pure LC Instrument

The MagNA Pure LC System is a benchtop system for the flexible isolation and purification of nucleic acids from a variety of sample materials. The core of the system is a reliable robotic system, processing up to 32 different samples in one batch. The system is based on the proven magnetic-bead technology, provided in dedicated high-quality reagents and kits. User-friendly software protocols adapt to specific sample requirements. In addition, the system has an option for automated, programmable pipetting of post-isolation PCR setup.

- **Automatically isolate any type of nucleic acid**
  The magnetic bead technology used in the MagNA Pure LC System, combined with dedicated kits, enables consistent isolation of high-quality DNA, RNA, or mRNA for all demanding nucleic acid research applications.

- **Process samples from a wide variety of sources**
  Easily isolate nucleic acids from whole blood, white blood cells, bacteria, tissue, and other difficult samples with the MagNA Pure LC Instrument.

- **Experience fast isolation and easy setup**
  Simple, software-guided handling steps launch the automated isolation procedure, processing up to 32 samples in as little as 60 minutes.

- **Rely on our high-quality reagents**
  High-quality kits and accessories provide consistent results that guarantee your success.

- **Let the instrument set up your experiment**
  Following nucleic acid isolation, the unique, programmable post-elution unit of the MagNA Pure LC Instrument automatically pipets sample and reagents for downstream PCR reactions, for example, with the LightCycler® System.

- **Experience outstanding reproducibility and scalability**
  Obtain consistent yield and recover your purified nucleic acids with higher efficiency, regardless of the volume of starting material – a must for today’s nucleic acid research.
MagNA Pure Compact Instrument

The MagNA Pure Compact System is the automated benchtop solution for nucleic acid purification. With its small instrument size, extensive integrated features, and a sample throughput of one to eight samples per run, the instrument meets the demanding nucleic acid isolation needs of research laboratories with low to medium sample throughput.

- **Conserve valuable laboratory space with the small footprint**
- **Obtain high-quality nucleic acids**
  from diverse sample types with proven reagent chemistry.
- **Incorporate a variety of protocols**
  using different specimen and elution volumes for a broad range of sample materials.
- **Save time through easy setup with prefilled reagents and disposables**
- **Eliminate contamination**
  with prefilled reagents and disposables, an integrated HEPA filter, and synchronized stage movement.
- **Ensure isolation success**
  with a sensor for tip loss, clot, and cartridge detection.
- **Track sample identification**
  with the supplied bar-code scanner.
- **Simplify documentation**
  via host connectivity.
- **Navigate easily**
  with the intuitive software and touch-screen monitor.
Magnetic Bead Technology results in highest purification quality and efficiency

Magnetic particles are the material of choice for automated nucleic acid purification. Using this technology, the MagNA Pure LC Instrument and the MagNA Pure Compact Instrument perform all steps of the procedure (sample uptake, lysis, binding to magnetic particles, wash steps, and elution) in specially designed pipette tips (Figure 59). Centrifugation and any other manual steps during the purification protocol are completely eliminated. Furthermore, the application of the magnetic bead technology within the MagNA Pure LC Instrument and the MagNA Pure Compact Instrument eliminates the need for vacuum pumps or tubing and the risk of cross contamination.

![Figure 59: Overview of nucleic acid isolation and purification with the MagNA Pure System.](image)

To learn more about MagNA Pure LC and the MagNA Pure Compact Instruments, visit [www.magnapure.com](http://www.magnapure.com)
Premium Performance Products for PCR and RT-PCR

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Master Mixes 230
Real-Time PCR Instruments 231
Reagents for Real-Time PCR 232
Introduction

The key to successful PCR is the preparation of high-purity nucleic acid templates. Some nucleic acids, as well as other substances, may interfere with PCR. In particular, clinical samples like blood and body fluids may contain inhibitory factors such as EDTA, heparin and porphyrins (hemoglobin).

Nucleic acid purification should:
- Allow the isolation of intact DNA or RNA templates from numerous biological sources and at various scales
- Guarantee the complete removal of interfering nucleic acids and low molecular weight components
- Involve minimal exposure to chemical hazards (e.g., phenol) that affect the quality of the nucleic acid
- Process a large number of samples quickly and conveniently

Roche Applied Science provides an extensive product line to meet all of your amplification needs. Although all of our products will perform in a broad array of applications, you can leverage products’ individual characteristics for your specific applications.

This chapter provides an overview on our innovative high quality products for RT-PCR and PCR. These products have been function tested to perform optimally with the nucleic acid purification products listed in this manual.

Choose Roche Applied Science RT-PCR and PCR products to obtain:
- Maximum performance and reproducibility
- Optimal results, whether you are:
  - Amplifying short, medium or long DNA fragments
  - Cloning with PCR
  - Attempting to detect trace quantities of mRNA

Where to get more information on our PCR and RT-PCR products

To learn more about these products and their uses, your Roche Applied Science representative can provide the following information:
- PCR Applications Manual (also available on our web site: www.roche-applied-science.com/prod_inf/manuals/pcr_man/start.html)
- Biochemicals Catalog
- Biochemica newsletters, (also available on our web site: www.roche-applied-science.com)
- Brochure “Tools for Amplification”.
PCR Product Selection Guide

Roche Applied Science provides an extensive product line to meet all of your amplification needs. With more than 50 years of enzyme-purification expertise, Roche Applied Science has mastered the science of enzyme blending to bring you premium performance PCR and RT-PCR products.

- **Set your sights on the new standard**
  Improved results are seen on upgrading to hot start PCR with FastStart Taq DNA Polymerase, and FastStart High Fidelity PCR System.

- **Insist on excellent fidelity**
  Robust buffer systems facilitate outstanding yields and accuracy without optimization.

- **Conquer difficult assays**
  Roche Applied Science’s optimally designed systems enable the amplification of the most difficult templates, or multiple fragments.

- **Amplify longer fragments**
  More full-length product – up to 35 kb – is obtained with higher fidelity and yields from complex genomic DNA.
RT-PCR Product Selection Guide

Choose Transcriptor for your RT-PCR application and increase sensitivity in quantitative RT-PCR. Simultaneously reverse transcribe rare and abundant RNA – without distorting gene expression levels. Save your RNA from degradation using the Protector RNase Inhibitor included in all Transcriptor kits.

Transcriptor First Strand cDNA Synthesis Kit
- **Reverse transcribe rare and abundant RNA** without altering gene expression levels.
- **Use GC-rich RNAs** at +55°C together with an anchored-oligo(dT)₈ primer to generate full-length cDNAs up to 14 kb.
- **Benefit from a kit that contains all reaction components**, specifically designed for use with real-time PCR instruments.

Transcriptor High Fidelity cDNA Synthesis Kit
- **Achieve 7-fold higher fidelity** compared to commonly used reverse transcriptases (see Figures 61 and 62).
- **Perform full-length cDNA synthesis in only 10 minutes.**

Transcriptor One Step RT-PCR Kit
- **Detect as little as 1 fg of total RNA or 1 copy of viral RNA** (*in vitro* transcripts).
- **Minimize pipetting steps and prevent contamination.**
- **Use a premixed enzyme blend** and optimized buffer that includes PCR grade dNTPs and hot start components.

Product Selection Guide
A comprehensive portfolio for cDNA synthesis and RT-PCR

<table>
<thead>
<tr>
<th></th>
<th>Two-Step RT-PCR</th>
<th>One-Step RT-PCR</th>
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<tr>
<td><strong>Product Size</strong></td>
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<td>up to 14 kb</td>
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<tr>
<td><strong>Yield</strong></td>
<td>****</td>
<td>****</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>****</td>
<td>****</td>
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<td><strong>Difficult Templates</strong></td>
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<td>45 – 55°C</td>
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<td><strong>Full-Length cDNA</strong></td>
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<td>****</td>
</tr>
<tr>
<td><strong>Performance in qRT-PCR</strong></td>
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<td>****</td>
</tr>
<tr>
<td><strong>Proofreading Activity</strong></td>
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<td>yes</td>
</tr>
</tbody>
</table>

- Transcriptor Reverse Transcriptase
- Transcriptor High Fidelity cDNA Synthesis Kit
- Transcriptor First Strand cDNA Synthesis Kit
- Transcriptor One-Step RT-PCR Kit
Choose Roche Applied Science’s dNTPacks, convenient products that combine PCR-Grade Nucleotides, thermostable enzymes and enzyme blends, and all associated components such as buffers and PCR-enhancing additives. Our PCR Grade Nucleotides are assayed for function in RT-PCR, ensuring optimal performance of all components. Each dNTPack contains the additive-free sodium salt nucleotides as a ready-to-use mix (10 mM of each dNTP).

- **Profit from best performance**  
  Superior enzymes, combined with a mix of ultrapure PCR-Grade Nucleotides, ensure highest sensitivity and performance of amplification reactions.

- **Safeguard your precious reaction components**  
  The extensive investment in generating template material should not be risked by using nucleotides from another supplier.

- **Simplify ordering**  
  dNTPacks provide everything you need for PCR in one convenient package.

- **Benefit from an attractive price**  
  Thermostable DNA polymerases and premixed solutions of PCR-Grade Nucleotides are provided in one economical package.
Master Mixes

Combine all the benefits of our amplification products with all the benefits of our PCR-Grade Nucleotides in an ultimate convenient format. Roche Applied Science’s Master mixes are ready-to-use, double-concentrated solutions that contain all the reagents (except PCR primers and template) needed for running PCR: Polymerase or polymerase blend, magnesium chloride, double-concentrated reaction buffer, and nucleotides (dATP, dCTP, dGTP, dTTP, 0.4 mM each).

- **Simplify PCR setup**
  Only two pipetting steps are needed before starting PCR.

- **Minimize the risk of contamination**
  The ready-to-use reaction mix reduces potential contacts to contamination sources.

- **Enjoy convenient packaging options**
  Master mixes can be obtained in 50 ml quantities designed for high throughput applications, or in multiple vials containing smaller volumes.

- **Save valuable time**
  No thawing required because of +4°C stability.

- **Insist on room temperature stability**
  Roche Applied Science’s master mixes are compatible with robotics pipetting stations because of tested stability at room temperature.

<table>
<thead>
<tr>
<th>Length</th>
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<th>High Fidelity PCR</th>
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<tbody>
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</tr>
<tr>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
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<td></td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>1 kb</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Basic PCR</th>
<th>High Fidelity PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>**</td>
<td>*****</td>
</tr>
<tr>
<td>Sensitivity</td>
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<td>*****</td>
</tr>
<tr>
<td>Robustness</td>
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</tr>
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<td>1 x</td>
</tr>
<tr>
<td>Carryover Prevention</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

* compared to Taq DNA Polymerase
Real-Time PCR Instruments Available from Roche Applied Science

Real-time PCR offers an alternative method for both qualitative and quantitative analysis. This type of analysis allows the amplification and fluorescent detection steps to be performed by a single instrument in a single tube with data recorded online. A real-time PCR instrument measures the accumulation of PCR products during amplification with fluorescent dyes. Because PCR itself and the detection of PCR products occur in the same reaction (vessel), this set-up is also called “homogeneous PCR”. The LightCycler® System incorporates several features that make it the ideal tool for qualitative and quantitative PCR as well as mutation analysis in general laboratory applications. It includes instrumentation, software, reagents, technical support, and application-specific kits.

Two real-time PCR systems are available from Roche Applied Science:

**The LightCycler® Carousel-Based System** (LightCycler® 2.0 Instrument, Cat. No.: 03 531 414 201).

The LightCycler® 2.0 Instrument is optimized for two fluorescence detection formats: SYBR Green I and HybProbe probes. In addition, the instrument supports a wide variety of other fluorescence detection formats, such as monochrome SimpleProbe probes, hydrolysis probes, and other formats based on FRET (fluorescence resonance energy transfer).

**The LightCycler® 480 System** (Cat. No. 04 640 268 001 → 96 well; Cat. No. 04 545 885 001 → 384 well) is a modular online PCR device for qualitative or quantitative detection of nucleic acids, mutation screening and genotyping. It meets the needs of a broad range of scientific applications in genomics research, such as array validation, gene-knockdown studies, and SNP analysis.

Offering the sensitivity and accuracy one has come to expect only from Roche Applied Science’s LightCycler® Carousel-Based System, the LightCycler® 480 Real-time PCR System goes one step further providing enhanced 96- or 384-multiwell throughput.

The LightCycler® 480 System setup enables the use of all current probe formats (e.g., SYBR Green I, HybProbe probes, SimpleProbe probes, hydrolysis probes and ResoLight dye for high resolution melting).

For even higher throughputs (up to 1536 reactions in one run) and smaller reaction volumes (0.5-2μl), a separate plate-based instrument, the LightCycler® 1536 System, is available.

For details concerning the instruments, reagents and software please refer to [http://www.lightcycler.com](http://www.lightcycler.com), [http://www.lightcycler480.com](http://www.lightcycler480.com) and [www.lightcycler1536.com](http://www.lightcycler1536.com)

ResoLight is a trademark of Roche
Reagents for Real-Time PCR

Roche Applied Science offers tailor-made reagents for all real-time PCR applications. For reagents developed for the LightCycler® Instrument please refer to our website www.lightcycler.com. If you are still using a different real-time PCR instrument than the LightCycler® Instrument, you can choose from our list of dedicated reagents. For details please refer to: www.roche-applied-science.com.

Discover also the powerful combination of the ProbeFinder Software, the Universal ProbeLibrary, the Transcriptor First Strand cDNA Synthesis Kit, and the FastStart TaqMan® Probe Master. Revolutionize the way you design and perform real-time qPCR assays on various real-time PCR instruments. For qPCR experiments on instruments requiring normalization with Rox reference dye the FastStart Universal Probe Master (Rox) and the FastStart Universal SYBR Green Master (Rox) are available from Roche Applied Science. For more information, please visit www.roche-applied-science.com/qpcr.
Appendix

Tips for Handling Nucleic Acids 234
Conversion Tables and Formulas 236
Buffers and Gels for Electrophoresis 240
Other Useful Information 242
I. Tips for Handling Nucleic Acids

Tips for handling nucleic acids

Success of most procedures in molecular biology depends upon the purity and integrity of nucleic acids. Below are some general guidelines for handling nucleic acids. These tips are not intended to cover these topics in detail, but to identify important areas which should be considered when performing experimental procedures.

Precautions for handling DNA

Handling fresh and stored material before extraction of DNA

For the isolation of genomic DNA from cells or tissues, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at –70°C. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases. Follow this procedure for both genomic DNA and plasmid DNA.

Storage of DNA

Store genomic DNA at +2 to +8°C. Storing genomic DNA at –15 to –25°C can cause shearing of DNA, particularly if the DNA is exposed to repeated freeze-thaw cycles. Plasmid DNA and other small circular DNAs can be stored at +2 to +8°C or at –15 to –25°C.

Genomic and plasmid DNA can also be stored in small aliquots. Repeated use of a single sample may lead to shearing.

Drying, dissolving and pipetting DNA

Avoid overdrying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution. Plasmid DNA and other small circular DNAs can be vacuum dried.

To help dissolve the DNA, carefully invert the tubes several times after adding buffer and or tap the tube gently on the side. Alternatively let the DNA stand in buffer overnight at +2 to +8°C. Minimize vortexing of genomic DNA since this can cause shearing.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA. Regular pipette tips pose no problem for plasmid DNA and other small circular DNAs.

Strategies to produce an RNase-free environment

Gloves and contact

When working with RNA, wear gloves at all times. After putting on gloves, avoid touching contaminated surfaces and equipment with the gloved hands. Even if all the reagents have been decontaminated, RNases can be reintroduced by contact with ungloved hands or with unfiltered air.

Equipment and disposable items

Use sterile, disposable plasticware whenever possible. These require no treatment and are considered to be RNase-free. Electrophoresis tanks for RNA analysis can be cleaned by wiping them with a solution of SDS (1%), rinsing with water, then rinsing with absolute ethanol, and finally soaking them in 3% H₂O₂ for 10 min. Rinse tanks with DEPC-treated and autoclaved water before use (see below).
Glass- and plasticware
Treat glass- and plasticware with RNase inactivating agents. Glassware should be baked at $+180^\circ C$ for at least 4 hours. Autoclaving glassware alone is not sufficient to eliminate RNases from your experiments. Plasticware should be soaked (2 h, $+37^\circ C$) in 0.1 M NaOH/1 mM EDTA (or absolute ethanol with 1% SDS), rinsed with DEPC (diethyl pyrocarbonate) or DMPC\(^1\) treated water and heated to $+100^\circ C$ for 15 min in an autoclave.

To treat water with DEPC (DMPC) first incubate it with DEPC (2 h, $+37^\circ C$) and then autoclave it to hydrolyze any unreacted DEPC.

Workspace and working surfaces
Designate a special area for RNA work only. Treat surfaces of benches and glassware with commercially available RNase inactivating agents. Also, wipe benches with 100% ethanol each time prior to use, in order to rid the area of microorganisms.

Reagents
Whenever possible purchase reagents that are free of RNases. Separate reagents used for RNA work from ‘general use reagents’ in the laboratory. All solutions, except Tris buffers, should be treated with 0.1% DEPC (DMPC) overnight at room temperature and then autoclaved. Autoclaving hydrolys and destroys unreacted DEPC (DMPC). Alternatively, solutions can be made with DEPC-treated and autoclaved water in RNase-free glassware.

Tris reacts chemically with DEPC (DMPC), and therefore, solutions of Tris cannot be made RNase-free using DEPC (DMPC). Dedicate one bottle of Tris for RNA work only. Use baked spatulas and glassware and DEPC (DMPC) treated water for making the buffers.

Autoclaving without treatment with DEPC (DMPC) is not sufficient for inactivating RNases.

Precautions for handling RNA
Handling of fresh and stored material before extraction of RNA
Extract RNA as quickly as possible after obtaining samples. For the best results, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at $–70^\circ C$. RNA in inadequately maintained samples can be degraded by intracellular nucleases, especially in tissues that are rich in nucleases (such as spleen and pancreas).

RNase inhibitors
RNase inhibitors can be used to protect RNA from degradation both during isolation and purification and also in downstream applications such as reverse transcription into cDNA by RT-PCR, \textit{in vitro} RNA transcription/translation reactions and RNA-dependent \textit{in vitro} functional assays.

Protector RNase Inhibitor is one of the best characterized RNase inhibitor that inhibits a wide spectrum of RNases (RNase A, RNase B, RNase T1, RNase T2, RNase 1) offering the best protection of precious RNA samples. Protector RNase Inhibitor is fully active over a broad temperature range of 25 to $+55^\circ C$. Even at $+60^\circ C$ some RNase inhibition is still measured. This is advantageous when performing reverse transcription reactions at elevated temperatures to overcome secondary structure in RNA. To keep the inhibitor active, avoid temperatures $>60^\circ C$ or solutions containing strong denaturing agents such as SDS or urea and maintain reducing conditions (1 mM DTT). To protect difficult RNA samples the amount of Protector RNase Inhibitor could be increased up to 16 times the standard concentration without interfering with the performance of enzymes used in the assay.

Other inhibitors include Macaloid and Vanadyl-ribonucleoside complexes.

\(^1\) Dimethyl pyrocarbonate, a less toxic alternative to DEPC that can be used in the same manner as DEPC.
Storage of RNA
Store RNA at –70° to –80°C, as aliquots in ethanol or isopropanol. Most RNA is relatively stable at this temperature. Centrifuge the RNA and resuspend in the appropriate RNase-free buffer before use.

Drying, dissolving and pipetting RNA
RNA can be dried briefly at +37°C or in a vacuum oven. When working with RNA, place all samples on ice. For the reasons mentioned above, RNA is very susceptible to degradation when left at room temperature.

Dissolve RNA by adding RNase-free buffer or water, then standing the tube on ice for 15 min. Gently tap the tube or use vortexing with caution.

Temperature sensitivity
Although DNA is relatively stable at elevated temperatures (+100°C), most RNA is not (except for short RNA probes which are stable for 10 min at +100°C). Therefore avoid high temperatures (>65°C) since these affect the integrity of the RNA. Instead, to melt out secondary structures, heat RNA to +65°C for 15 min in the presence of denaturing buffers.

II. Conversion Tables and Formulas

Metric prefixes (International System)

<table>
<thead>
<tr>
<th>Bigger</th>
<th>Prefix</th>
<th>Multiplication factor</th>
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</thead>
<tbody>
<tr>
<td>T</td>
<td>tera</td>
<td>10^{12}</td>
</tr>
<tr>
<td>G</td>
<td>giga</td>
<td>10^{9}</td>
</tr>
<tr>
<td>M</td>
<td>mega</td>
<td>10^{6}</td>
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<tr>
<td>k</td>
<td>kilo</td>
<td>10^{3}</td>
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<table>
<thead>
<tr>
<th>Smaller</th>
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<td>milli</td>
<td>10^{-3}</td>
</tr>
<tr>
<td>μ</td>
<td>micro</td>
<td>10^{-6}</td>
</tr>
<tr>
<td>n</td>
<td>nano</td>
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<tr>
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<td>pico</td>
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<tr>
<td>f</td>
<td>femto</td>
<td>10^{-15}</td>
</tr>
<tr>
<td>a</td>
<td>atto</td>
<td>10^{-18}</td>
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Moles, molar, molarity

<table>
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<th>Term</th>
<th>Symbol</th>
<th>Meaning</th>
<th>Example</th>
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<tbody>
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<td>mol</td>
<td>Absolute amount of a substance</td>
<td>1 mol = 6.022 x 10^{23} molecules</td>
</tr>
<tr>
<td>Molar or Molarity</td>
<td>mol/l or M</td>
<td>Concentration of a substance in a liquid</td>
<td>1 mol/l = 6.022 x 10^{23} molecules/liter</td>
</tr>
</tbody>
</table>

Example of molar equivalence

0.2 pmol in 100 μl means a concentration of 2 nM.
Example how to calculate: 0.2 pmol in 100 μl = 2 pmol in 1 ml = 2 nmol in 1 l.
Physical conversions and formulas

**Centrifugal force conversion** *(Dyson, 1991)*

$$\text{RCF} = (1.11 \times 10^{-5}) \times (\text{rpm})^2 \times r$$

RCF = relative centrifugal force (x g; where $g = 980 \text{ cm x sec}^{-2}$)

rpm = revolutions per minute

r = radius of rotor in mm

Nucleic acid data

Conversions

**Spectral constants for nucleotides** *(Adapted from Sambrook, et al., 1989)*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular weight (MW)</th>
<th>$\lambda_{\text{max}}$ (pH 7.0)</th>
<th>Absorbance at $\lambda_{\text{max}}$ (1 M solution)</th>
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</thead>
<tbody>
<tr>
<td>ATP</td>
<td>507.2</td>
<td>259</td>
<td>15,400</td>
</tr>
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<td>dGTP</td>
<td>507.2</td>
<td>253</td>
<td>13,700</td>
</tr>
<tr>
<td>UTP</td>
<td>484.2</td>
<td>260</td>
<td>10,000</td>
</tr>
<tr>
<td>dTTP</td>
<td>482.2</td>
<td>267</td>
<td>9,600</td>
</tr>
</tbody>
</table>

Molar concentration of nucleic acid = (observed absorbance at $\lambda_{\text{max}}$) ÷ absorbance at $\lambda_{\text{max}}$ for 1 M solution.

**Spectrophotometric equivalents**

<table>
<thead>
<tr>
<th>1 $A_{260}$ unit</th>
<th>Nucleic acid</th>
<th>Amount</th>
<th>Molarity (in nucleotides)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>double-stranded DNA</td>
<td>50 µg/ml</td>
<td>0.15 mM</td>
</tr>
<tr>
<td></td>
<td>single-stranded DNA</td>
<td>33 µg/ml</td>
<td>0.10 mM</td>
</tr>
<tr>
<td></td>
<td>single-stranded RNA</td>
<td>40 µg/ml</td>
<td>0.11 mM</td>
</tr>
<tr>
<td></td>
<td>oligonucleotide*</td>
<td>20 – 30 µg/ml</td>
<td>0.06 – 0.09 mM</td>
</tr>
</tbody>
</table>

* For exact determination of the molecular weight, see table “Conversions between weight and molarity of various DNAs” page 223.

Calculations

**Determining purity of nucleic acid preparations**

For pure DNA: $A_{260}/A_{280} \geq 1.8$

For pure RNA: $A_{260}/A_{280} \geq 2.0$

An $A_{260}/A_{280}$ ratio of <1.8 (DNA) or <2.0 (RNA) means the nucleic acid preparation contains contaminations (e.g., protein), or phenol.
Calculating molecular weight of nucleic acids (Adapted from Ausubel et al., 1988)

<table>
<thead>
<tr>
<th>For molecular weight of</th>
<th>Use this calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA base pair (sodium salt)</td>
<td>1 base pair = 665 daltons</td>
</tr>
<tr>
<td>double-stranded DNA molecule</td>
<td>(number of base pairs) x (665 daltons/base pair)</td>
</tr>
<tr>
<td>single-stranded DNA molecule</td>
<td>(number of bases) x (325 daltons/base)</td>
</tr>
<tr>
<td>single-stranded RNA molecule</td>
<td>(number of bases) x (340 daltons/base)</td>
</tr>
<tr>
<td>oligonucleotide</td>
<td>For dephosphorylated oligonucleotides: [((\text{number of } A \times 312.2) + (\text{number of } G \times 328.2) + (\text{number of } C \times 288.2) + (\text{number of } T \times 303.2)) - 61] For phosphorylated oligonucleotides: [((\text{number of } A \times 312.2) + (\text{number of } G \times 328.2) + (\text{number of } C \times 288.2) + (\text{number of } T \times 303.2)) + 17]</td>
</tr>
</tbody>
</table>

Conversions between picomoles and micrograms of DNA

For double-stranded DNA (dsDNA):

<table>
<thead>
<tr>
<th>To convert</th>
<th>Calculate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pmol to μg</td>
<td>[\text{pmol} \times N \times \frac{660 \text{pg}}{1 \text{pmol}} \times \frac{1 \mu g}{10^6 \text{pg}} = \mu g]</td>
</tr>
<tr>
<td>μg to pmol</td>
<td>[\mu g \times \frac{10^6 \text{pg}}{1 \mu g} \times \frac{\text{pmol}}{660 \text{pg}} = \text{pmol}]</td>
</tr>
</tbody>
</table>

* N = number of base pairs in DNA; 660, average molecular weight of a base pair.

For single-stranded DNA (ssDNA):

<table>
<thead>
<tr>
<th>To convert</th>
<th>Calculate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pmol to μg</td>
<td>[\text{pmol} \times N \times \frac{330 \text{pg}}{1 \text{pmol}} \times \frac{1 \mu g}{10^6 \text{pg}} = \mu g]</td>
</tr>
<tr>
<td>μg to pmol</td>
<td>[\mu g \times \frac{10^6 \text{pg}}{1 \mu g} \times \frac{\text{pmol}}{330 \text{pg}} \times \frac{1}{N} = \text{pmol}]</td>
</tr>
</tbody>
</table>

* N = number of nucleotides in DNA; 330, average molecular weight of a nucleotide

Calculating moles of ends

- Moles of ends for double-stranded DNA molecule:
  \[2 \times \text{grams of DNA} / (\text{MW in daltons})\]

- Picomoles of ends per microgram of double-stranded DNA:
  \[2 \times 10^6 / (660 \times \text{number of bases})\]

- Moles of ends generated by restriction endonuclease cleavage, circular DNA molecule:
  \[2 \times (\text{moles of DNA}) \times (\text{number of sites})\]

- Moles of ends generated by restriction endonuclease cleavage, linear DNA molecule:
  \[[2 \times (\text{moles of DNA}) \times (\text{number of sites})] + [2 \times (\text{moles of DNA})]\]
Conversions between weight and molarity of various DNAs

<table>
<thead>
<tr>
<th>Type</th>
<th>Size</th>
<th>pmol/μg</th>
<th>Molecules/μg</th>
<th>μg/pmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>oligonucleotide</td>
<td>20 nucleotides</td>
<td>152</td>
<td>9.1 x 10^{13}</td>
<td>0.0066</td>
</tr>
<tr>
<td>DNA</td>
<td>1000 bp</td>
<td>1.52</td>
<td>9.1 x 10^{11}</td>
<td>0.66</td>
</tr>
<tr>
<td>pUC18/19 DNA</td>
<td>2686 bp</td>
<td>0.57</td>
<td>3.4 x 10^{11}</td>
<td>1.77</td>
</tr>
<tr>
<td>pBR322 DNA</td>
<td>4361 bp</td>
<td>0.35</td>
<td>2.1 x 10^{11}</td>
<td>2.88</td>
</tr>
<tr>
<td>M13mp18/19 DNA</td>
<td>7250 bp</td>
<td>0.21</td>
<td>1.3 x 10^{11}</td>
<td>4.78</td>
</tr>
<tr>
<td>λ DNA</td>
<td>48,502 bp</td>
<td>0.03</td>
<td>1.8 x 10^{10}</td>
<td>32.01</td>
</tr>
</tbody>
</table>

Protein data

Conversion between DNA and protein

1 kb of DNA = 333 amino acids of coding capacity = a protein of 3.7 x 10^4 daltons

Conversion between protein molecular weight and absolute weight

<table>
<thead>
<tr>
<th>100 pmol</th>
<th>MW of protein [k daltons]</th>
<th>Amount in μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
### Running buffers for non-denaturing gel electrophoresis
*(Adapted from Brown, 1991)*

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Components (1 x buffer)</th>
<th>Recipe for 1 liter of 10 x buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBE*a</td>
<td>89 mM Tris-borate (pH 8.3) 2 mM Na₂EDTA</td>
<td>108.0 g Tris-base 55.0 g boric acid 9.3 g Na₂EDTA</td>
</tr>
<tr>
<td>(Tris-borate)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAE</td>
<td>40 mM Tris-acetate (pH 7.6) 1 mM Na₂EDTA</td>
<td>48.4 Tris-base 11.4 ml glacial acetic acid 20.0 ml 0.5 M Na₂EDTA (pH 8.0)</td>
</tr>
<tr>
<td>(Tris-acetate)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPE</td>
<td>89 mM Tris-phosphate 2 mM Na₂EDTA</td>
<td>108.0 g Tris-base 15.5 ml phosphoric acid (85%) 40.0 ml 0.5 M Na₂EDTA (pH 8.0)</td>
</tr>
<tr>
<td>(Tris-phosphate)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a) 0.5 x TBE can be used for agarose gels

1 x TBE is the standard running buffer for DNA separation in polyacrylamide gels.

b) Concentrated stock solutions of TBE tend to develop a precipitate when stored for long periods of time. Store at room temperature and discard any solution that develops a precipitate.

### Recommended agarose gel concentration for resolving linear DNA

<table>
<thead>
<tr>
<th>% Agarose</th>
<th>DNA size range (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.40</td>
<td>2000 – 30,000</td>
</tr>
<tr>
<td>0.75</td>
<td>1000 – 15,000</td>
</tr>
<tr>
<td>1.00</td>
<td>500 – 10,000</td>
</tr>
<tr>
<td>1.25</td>
<td>300 – 5000</td>
</tr>
<tr>
<td>1.50</td>
<td>200 – 4000</td>
</tr>
<tr>
<td>2.00</td>
<td>100 – 2500</td>
</tr>
</tbody>
</table>

### Gel loading buffers for non-denaturing agarose gel electrophoresis
*(Adapted from Brown, 1991)*

<table>
<thead>
<tr>
<th>Loading buffer</th>
<th>Recipe for 6 x buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBX (store at +15 to +25°C)</td>
<td>40% (w/v) Sucrose 0.25% (w/v) Bromphenol blue 0.25% (w/v) Xylene cyanol</td>
</tr>
<tr>
<td>FBX (store at +2 to +8°C)</td>
<td>15% (w/v) Ficoll 400 0.25% (w/v) Bromphenol blue 0.25% (w/v) Xylene cyanol</td>
</tr>
<tr>
<td>GBX (store at +2 to +8°C)</td>
<td>30% (v/v) Glycerol 0.25% (w/v) Bromphenol blue 0.25% (w/v) Xylene cyanol</td>
</tr>
</tbody>
</table>
Denaturing agarose gel electrophoresis

<table>
<thead>
<tr>
<th>Nucleic acid</th>
<th>Alkaline gels (McDonell et al., 1977)</th>
<th>Glyoxal gels (Bailey and Davidson, 1976)</th>
<th>Formaldehyde gels (Rueger et al., 1996)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principle</td>
<td>Gels run in alkaline buffer which denatures DNA</td>
<td>Nucleic acids are denatured with glyoxal prior to electrophoresis</td>
<td>Gels are run in the presence of formaldehyde to denature nucleic acids</td>
</tr>
<tr>
<td>Gel buffer</td>
<td>50 mM NaCl 1 mM Na₂EDTA (neutral pH, because at high temperatures alkaline pH hydrolyzes agarose)</td>
<td>10 mM sodium phosphate, pH 7.0 (neutral pH, because glyoxal dissociates from nucleic acids at pH &gt;8.0)</td>
<td>1 x MOPS* 2% (v/v) Formaldehyde</td>
</tr>
<tr>
<td>Running buffer</td>
<td>30 mM NaOH, 1 mM Na₂EDTA (Soak gel in running buffer 30 min before loading.)</td>
<td>Same as gel buffer (The running buffer must be circulated during electrophoresis.)</td>
<td>1 x MOPS*</td>
</tr>
<tr>
<td>Loading buffer</td>
<td>Resuspend DNA in: 50 mM NaOH 1 mM Na₂EDTA 3% (w/v) Ficoll 400 0.025% (w/v) Bromocresol green 0.025% (w/v) Xylene cyanol</td>
<td>Resuspend DNA/RNA in: 8 μl 1 M glyoxal 50% (v/v) DMSO 10 mM sodium phosphate, pH 7.0 Incubate at +50°C for 1 h; cool, then add: 2 μl 10 mM sodium phosphate, pH 7.0 50% (v/v) glycerol 0.4% (w/v) Bromophenol blue</td>
<td>Prepare freshly!! 250 μl formamide (deionized) 83 μl formaldehyde, 37% (w/v) 50 μl 10 x MOPS-buffer* 0.01% (w/v) Bromphenol blue Add RNA and incubate for 10 min at +65°C; then immediately cool on ice.</td>
</tr>
</tbody>
</table>

* 10 x MOPS: 200 mM Morpholinopropanesulfonic acid, pH 7.0 50 mM sodium acetate 10 mM Na₂EDTA

Non-denaturing polyacrylamide gels: Recommended polyacrylamide gel concentration for resolving dsDNA and behavior of marker dyes
(Adapted from Sambrook et al., 1989)

<table>
<thead>
<tr>
<th>% Acrylamide</th>
<th>DNA size range (bp)</th>
<th>Bromphenol Blue*</th>
<th>Xylene Cyanol*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>100 – 1000</td>
<td>100</td>
<td>460</td>
</tr>
<tr>
<td>5.0</td>
<td>75 – 500</td>
<td>65</td>
<td>260</td>
</tr>
<tr>
<td>8.0</td>
<td>50 – 400</td>
<td>45</td>
<td>160</td>
</tr>
<tr>
<td>12.0</td>
<td>35 – 250</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>15.0</td>
<td>20 – 150</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td>20.0</td>
<td>5 – 100</td>
<td>12</td>
<td>45</td>
</tr>
</tbody>
</table>

* Approximate size of DNA fragments (in base pairs) with which the dyes would migrate.
Denaturing polyacrylamide gels: Migration of marker dyes
(Adapted from Sambrook et al., 1989)

<table>
<thead>
<tr>
<th>% Acrylamide</th>
<th>Bromphenol Blue*</th>
<th>Xylene Cyanol*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>35</td>
<td>130</td>
</tr>
<tr>
<td>6.0</td>
<td>26</td>
<td>106</td>
</tr>
<tr>
<td>8.0</td>
<td>19</td>
<td>76</td>
</tr>
<tr>
<td>10.0</td>
<td>12</td>
<td>55</td>
</tr>
</tbody>
</table>

* Approximate size of DNA fragments (in nucleotides) with which the dyes would migrate. (Acrylamide: Bisacrylamide (w/w) should be 29:1.)

Agaroses and molecular weight markers
see inside back cover page.

Stains and tracker dyes

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>Application</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acridine Orange</td>
<td>301.8</td>
<td>DNA, RNA stain in gel electrophoresis</td>
<td>1 – 10 μg/ml in staining solution</td>
</tr>
<tr>
<td>Bromphenol Blue</td>
<td>670.0</td>
<td>Gel electrophoresis marker dye</td>
<td>0.01 – 0.05% in gel loading buffers</td>
</tr>
<tr>
<td>Ethidium bromide</td>
<td>394.3</td>
<td>Fluorescent dye for DNA/RNA in density gradients and electrophoresis gels</td>
<td>1 μg/ml in running buffer or staining solution</td>
</tr>
<tr>
<td>Xylene Cyanol</td>
<td>554.6</td>
<td>Gel electrophoresis marker dye</td>
<td>0.01 – 0.05% in gel loading buffers</td>
</tr>
</tbody>
</table>

IV. Other Useful Information

Length of rRNAs from various species
(Lewin, 1987)

<table>
<thead>
<tr>
<th>Species</th>
<th>RNA</th>
<th>Length (bases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>16 S rRNA</td>
<td>1542</td>
</tr>
<tr>
<td></td>
<td>23 S rRNA</td>
<td>2904</td>
</tr>
<tr>
<td>Yeast (S. cerevisiae)</td>
<td>18 S rRNA</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>28 S rRNA</td>
<td>3750</td>
</tr>
<tr>
<td>Drosophila (D. melanogaster)</td>
<td>18 S rRNA</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>28 S rRNA</td>
<td>4100</td>
</tr>
<tr>
<td>Mammal</td>
<td>18 S rRNA</td>
<td>1874</td>
</tr>
<tr>
<td></td>
<td>28 S rRNA</td>
<td>4718</td>
</tr>
</tbody>
</table>
Blood and its components
(For further information, see Czihak et al., Masseyeff et al.)

Blood

<table>
<thead>
<tr>
<th>blood cells</th>
<th>plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>erythrocytes</td>
<td>water</td>
</tr>
<tr>
<td>leukocytes</td>
<td>proteins</td>
</tr>
<tr>
<td>– lymphocytes</td>
<td>– albumins</td>
</tr>
<tr>
<td>– granulocytes</td>
<td>– globulins</td>
</tr>
<tr>
<td>– monocytes</td>
<td>– clotting factors</td>
</tr>
<tr>
<td>thrombocytes</td>
<td>carbohydrates</td>
</tr>
<tr>
<td></td>
<td>fats</td>
</tr>
<tr>
<td></td>
<td>electrolytes</td>
</tr>
</tbody>
</table>

Serum: Clear, yellowish fluid that separates from blood when it clots. Serum, unlike plasma, does not contain fibrinogen; serum also lacks several clotting factors present in plasma.

Buffy coat: Following centrifugation of whole blood, the buffy coat is the interface layer of material between the plasma-containing upper phase and the erythrocyte-containing lower phase. It is comprised of leukocytes and thrombocytes.

References


Casey, J. and Davidson, N. (1977) Nucleic Acids Res. 4, 1539

Czihak, G., Langer, H. and Ziegler, H., Biologie, Springer Verlag


Masseyeff, R.F., Albert, W.H. and Staines, N.A., Methods of Immunological Analysis, Cells and Tissues, VCH


Wallace, R.B. et al. (1979) Nucleic Acids Res. 6, 3543
Ordering Guide

Isolation and Purification of DNA  246
Isolation and Purification of RNA  247
MagNA Lyser Instrument and Accessories  248
Automated Isolation using the MagNA Pure LC Instrument  248
Automated Isolation using the MagNA Pure Compact Instrument  249
Companion Reagents for Isolating Nucleic Acids  250
## Isolation and Purification of DNA

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat. No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose Gel DNA Extraction Kit*</td>
<td>11 696 505 001</td>
<td>up to 100 purifications</td>
</tr>
<tr>
<td>for the elution of DNA from agarose gel slices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA Isolation Kit for Cells and Tissue*</td>
<td>11 814 770 001</td>
<td>10 isolations for</td>
</tr>
<tr>
<td>for the extraction of genomic DNA from cells and tissues ranging in size</td>
<td></td>
<td>400 mg tissue or</td>
</tr>
<tr>
<td>from 50 to 150 kb</td>
<td></td>
<td>5 x 10^7 cultured cells</td>
</tr>
<tr>
<td>DNA Isolation Kit for Mammalian Blood*</td>
<td>11 667 327 001</td>
<td>25 purifications</td>
</tr>
<tr>
<td>for the isolation of intact genomic DNA from mammalian whole blood, lymphocyte, or buffy coat samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Pure 96 UF Cleanup Kit*</td>
<td>04 422 694 001</td>
<td>2 x 96 purifications</td>
</tr>
<tr>
<td>for high-throughput purification of PCR products by ultrafiltration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Pure 96 UF Cleanup Plates*</td>
<td>04 422 716 001</td>
<td>10 x 96 reactions</td>
</tr>
<tr>
<td>for high-throughput purification of PCR products by ultrafiltration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Pure PCR Cleanup Micro Kit*</td>
<td>04 983 955 001</td>
<td>up to 50 purifications</td>
</tr>
<tr>
<td>for purification of products from PCR and other reactions</td>
<td></td>
<td>up to 200 purifications</td>
</tr>
<tr>
<td>High Pure PCR Product Purification Kit*</td>
<td>11 732 668 001</td>
<td>up to 50 purifications</td>
</tr>
<tr>
<td>for the purification of PCR reaction products</td>
<td></td>
<td>up to 250 purifications</td>
</tr>
<tr>
<td>High Pure PCR Template Preparation Kit*</td>
<td>11 796 828 001</td>
<td>up to 100 purifications</td>
</tr>
<tr>
<td>for isolating genomic nucleic acids for PCR, restriction enzymes, analysis, and Southern blotting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Pure Plasmid Isolation Kit*</td>
<td>11 754 777 001</td>
<td>up to 50 purifications</td>
</tr>
<tr>
<td>small scale “mini-preps” for sequencing, PCR, and cloning</td>
<td></td>
<td>up to 250 purifications</td>
</tr>
<tr>
<td>High Pure Viral Nucleic Acid Kit*</td>
<td>11 858 874 001</td>
<td>up to 100 purifications</td>
</tr>
<tr>
<td>for isolating viral DNA and RNA for PCR or RT-PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Pure Viral Nucleic Acid Kit Large Volume*</td>
<td>05 114 403 001</td>
<td>up to 40 purifications</td>
</tr>
<tr>
<td>for the isolation of viral nucleic acids for PCR and RT-PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Pure Viral Nucleic Acid Buffer Set*</td>
<td>12 011 875 001</td>
<td>up to 100 reactions</td>
</tr>
<tr>
<td>for the isolation of viral nucleic acids for PCR and RT-PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quick Spin Columns for radiolabeled DNA*</td>
<td>11 273 922 001</td>
<td>20 columns</td>
</tr>
<tr>
<td>Sephadex G-25</td>
<td>11 273 949 001</td>
<td>50 columns</td>
</tr>
<tr>
<td>Quick Spin Columns for radiolabeled DNA*</td>
<td>11 273 965 001</td>
<td>20 columns</td>
</tr>
<tr>
<td>Sephadex G-50</td>
<td>11 273 973 001</td>
<td>50 columns</td>
</tr>
<tr>
<td>mini Quick Spin DNA Columns*</td>
<td>11 814 419 001</td>
<td>50 columns</td>
</tr>
<tr>
<td>mini Quick Spin Oligo Columns*</td>
<td>11 814 397 001</td>
<td>50 columns</td>
</tr>
<tr>
<td>Genopure Plasmid Midi Kit*</td>
<td>03 143 414 001</td>
<td>up to 20 preparations</td>
</tr>
<tr>
<td>for medium-scale (midi) preparation of plasmid DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genopure Plasmid Maxi Kit*</td>
<td>03 143 422 001</td>
<td>up to 10 preparations</td>
</tr>
<tr>
<td>for large-scale (maxi) preparation of plasmid DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genopure Buffer Set for Low-Copy Number Plasmids*</td>
<td>04 634 772 001</td>
<td>up to 20 maxi preps or</td>
</tr>
<tr>
<td>for isolation of low-copy number plasmid DNA in combination with the Genopure Plasmid Kits</td>
<td></td>
<td>60 midi preps</td>
</tr>
<tr>
<td>Red Blood Cell Lysis Buffer</td>
<td>11 814 389 001</td>
<td>100 ml</td>
</tr>
<tr>
<td>for the preferential lysis of erythrocytes in human whole blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TriPure Isolation Reagent*</td>
<td>11 667 157 001</td>
<td>50 ml</td>
</tr>
<tr>
<td>for the simultaneous isolation of DNA, RNA, and denatured proteins from cells or tissues of human, plant, yeast, bacterial, or viral origin.</td>
<td></td>
<td>200 ml</td>
</tr>
</tbody>
</table>

* For life science research only. Not for use in diagnostic procedures.
+ For general laboratory use.
# Isolation and Purification of RNA

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat. No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Pure FFPE RNA Micro Kit+ for isolation of total RNA from formalin-fixed, paraffin-embedded tissue</td>
<td>04 823 125 001</td>
<td>up to 50 isolations</td>
</tr>
<tr>
<td>High Pure RNA Isolation Kit* for small scale preparations of total RNA (free of genomic DNA) from blood, cultured cells, yeast, and bacteria</td>
<td>11 828 665 001</td>
<td>up to 50 purifications</td>
</tr>
<tr>
<td>High Pure RNA Tissue Kit* for the isolation of total RNA from tissue</td>
<td>12 033 674 001</td>
<td>up to 50 isolations</td>
</tr>
<tr>
<td>High Pure RNA Paraffin Kit* for the isolation of total RNA from fresh-frozen and formalin-fixed, paraffin-embedded tissues</td>
<td>03 270 289 001</td>
<td>up to 100 purifications</td>
</tr>
<tr>
<td>High Pure Viral RNA Kit+ for the isolation of viral RNA for RT-PCR</td>
<td>11 858 882 001</td>
<td>up to 100 purifications</td>
</tr>
<tr>
<td>High Pure miRNA Isolation Kit* for purification of small or total RNA from cells, tissue, FFPE tissue sections</td>
<td>05 080 576 001</td>
<td>up to 50 isolations</td>
</tr>
<tr>
<td>mRNA Capture Kit* for the immobilization of poly(A+) RNA in a PCR tube, prior to reverse transcription</td>
<td>11 787 896 001</td>
<td>192 reactions</td>
</tr>
<tr>
<td>mRNA Isolation Kit* for the affinity isolation of poly(A+) RNA</td>
<td>11 741 985 001</td>
<td>at least 70 μg mRNA</td>
</tr>
<tr>
<td>mRNA Isolation Kit for Blood/Bone Marrow* for the isolation of mRNA from whole blood or bone marrow lysates, preserved with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow</td>
<td>11 934 333 001</td>
<td>30–100 isolations</td>
</tr>
<tr>
<td>RNA/DNA Stabilization Reagent for Blood/Bone Marrow* for instantaneous stabilization of DNA and RNA in blood or bone marrow samples prior to isolation mRNA or DNA (used with the mRNA Isolation Kit for Blood/Bone Marrow)</td>
<td>11 934 317 001</td>
<td>500 ml</td>
</tr>
<tr>
<td>Quick Spin Columns for radiolabeled RNA* Sephadex G-25</td>
<td>11 273 990 001</td>
<td>20 columns</td>
</tr>
<tr>
<td>Quick Spin Columns for radiolabeled RNA* Sephadex G-50</td>
<td>11 274 015 001</td>
<td>20 columns</td>
</tr>
<tr>
<td>mini Quick Spin RNA Columns*</td>
<td>11 814 427 001</td>
<td>50 columns</td>
</tr>
<tr>
<td>Red Blood Cell Lysis Buffer for the preferential lysis of erythrocytes in human whole blood</td>
<td>11 814 389 001</td>
<td>100 ml</td>
</tr>
<tr>
<td>Streptavidin Magnetic Particles</td>
<td>11 641 778 001</td>
<td>2 ml</td>
</tr>
<tr>
<td></td>
<td>11 641 786 001</td>
<td>10 ml</td>
</tr>
<tr>
<td>TriPure Isolation Reagent* for the simultaneous isolation of DNA, RNA, and denatured proteins from cells or tissues of human, plant, yeast, bacterial, or viral origin.</td>
<td>11 667 157 001</td>
<td>50 ml</td>
</tr>
<tr>
<td></td>
<td>11 667 165 001</td>
<td>200 ml</td>
</tr>
</tbody>
</table>

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+ For general laboratory use.
MagNA Lyser Instrument and Accessories

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Cat. No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Lyser Instrument</td>
<td>Automated homogenization of tissue samples</td>
<td>03 358 968 001</td>
<td>1 instrument plus accessories</td>
</tr>
<tr>
<td></td>
<td></td>
<td>03 358 976 001</td>
<td></td>
</tr>
<tr>
<td>MagNA Lyser Rotor</td>
<td>Holds up to 16 sample tubes for tissue homogenization</td>
<td>03 359 093 001</td>
<td>1 rotor</td>
</tr>
<tr>
<td>MagNA Lyser Rotor Cooling Block</td>
<td>Houses the MagNA Lyser Rotor to maintain the temperature of the samples at +2 to +8°C</td>
<td>03 359 085 001</td>
<td>1 cooling block</td>
</tr>
<tr>
<td>MagNA Lyser Green Beads</td>
<td>Specially designed ceramic beads to achieve optimal homogenization of various sample materials</td>
<td>03 358 941 001</td>
<td>100 tubes (prefilled with ceramic beads)</td>
</tr>
</tbody>
</table>

Automated Isolation using the MagNA Pure LC Instrument

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Cat. No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure LC Instrument</td>
<td>Robotic workstation for fully automated nucleic acid preparation and filling of LightCycler® Capillaries, 96-well PCR plates, and tubes suitable for the most commonly used PCR instruments</td>
<td>12 236 931 001</td>
<td>1 instrument plus accessories</td>
</tr>
<tr>
<td>MagNA Pure LC DNA Isolation Kit I</td>
<td>Ready-to-use reagents for the isolation of high-quality genomic DNA from whole blood, white blood cells, peripheral blood lymphocytes, and cultured cells, using the MagNA Pure LC Instrument.</td>
<td>03 003 990 001</td>
<td>1 kit (192 reactions)</td>
</tr>
<tr>
<td>MagNA Pure LC DNA Isolation Kit II (Tissue)</td>
<td>Ready-to-use reagents for the isolation of high-quality, intact genomic DNA from a wide variety of human and animal tissue samples, using the MagNA Pure LC Instrument.</td>
<td>03 186 229 001</td>
<td>1 kit (192 reactions)</td>
</tr>
<tr>
<td>MagNA Pure LC DNA Isolation Kit III (Bacteria &amp; Fungi)</td>
<td>Ready-to-use reagents for the isolation of high-quality, intact bacterial or fungal DNA from the most difficult-to-process research sample materials, using the MagNA Pure LC Instrument.</td>
<td>03 264 785 001</td>
<td>1 kit (192 reactions)</td>
</tr>
<tr>
<td>MagNA Pure LC DNA Isolation Kit – Large Volume</td>
<td>Ready-to-use reagents for the purification of genomic DNA from large amounts of whole blood (up to 1 ml), blood cells, or culture cells (up to 5 x 10⁶), using the MagNA Pure LC Instrument.</td>
<td>03 310 515 001</td>
<td>1 kit (96 – 288 reactions)</td>
</tr>
<tr>
<td>MagNA Pure LC RNA Isolation Kit</td>
<td>Ready-to-use reagents for the purification of viral nucleic acid from serum, plasma and whole blood, using the MagNA Pure LC Instrument</td>
<td>03 038 505 001</td>
<td>1 kit (192 reactions)</td>
</tr>
<tr>
<td>MagNA Pure LC RNA Isolation Kit – Large Volume</td>
<td>Ready-to-use reagents for the purification of viral nucleic acids from large amounts (up to 1 ml) of serum and plasma, using the MagNA Pure LC Instrument</td>
<td>03 264 793 001</td>
<td>1 kit (192 reactions)</td>
</tr>
</tbody>
</table>

Reagent Kits for Isolation of total RNA

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Cat. No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure LC RNA Isolation Kit – High Performance</td>
<td>Ready-to-use reagents, developed to maximize yield of purified total RNA with superior quality isolated from blood, blood cells or culture cells, using the MagNA Pure LC Instrument</td>
<td>03 542 394 001</td>
<td>1 kit (192 reactions)</td>
</tr>
</tbody>
</table>

* CE marked /for USA for laboratory use
* For general laboratory use.
### Automated Isolation using the MagNA Pure LC Instrument, continued

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Cat. No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure LC RNA Isolation Kit III+ (Tissue)</td>
<td>Ready-to-use reagents for the purification of total RNA from human and animal frozen (−80°C) tissues or other pre-treated (e.g., RNA later® tissues (10 mg tissue or less), as well as paraffin-embedded tissue sections, using the MagNA Pure LC Instrument</td>
<td>03 330 591 001</td>
<td>1 kit (192 reactions)</td>
</tr>
</tbody>
</table>

### Reagent Kits for Isolation of mRNA

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Cat. No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure LC mRNA Isolation Kit I+ (Blood, Blood Cells)</td>
<td>Ready-to-use reagents for the isolation of high-quality and undegraded mRNA from whole blood, white blood cells, peripheral blood mononuclear cells, and culture cells, using the MagNA Pure LC Instrument</td>
<td>03 004 015 001</td>
<td>1 kit (192 reactions)</td>
</tr>
<tr>
<td>MagNA Pure LC mRNA Isolation Kit II+ (Tissue)</td>
<td>Ready-to-use reagents for the isolation of high-quality, intact mRNA from a wide variety of human and animal tissue samples, using the MagNA Pure LC Instrument.</td>
<td>03 172 627 001</td>
<td>1 kit (192 reactions)</td>
</tr>
<tr>
<td>MagNA Pure LC mRNA HS Kit</td>
<td>Ready-to-use reagents for the purification of mRNA from up to 1 x 10⁷ WBCs (white blood cells) or PBMCs (peripheral blood mononuclear cells), using the MagNA Pure LC Instrument</td>
<td>03 267 393 001</td>
<td>1 kit (192 reactions)</td>
</tr>
</tbody>
</table>

### Automated Isolation using the MagNA Pure Compact Instrument

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Cat. No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure Compact Instrument+</td>
<td>Automated nucleic acid isolation for a broad range of applications</td>
<td>03 731 146 001</td>
<td>1 instrument including internal PC with touch-screen monitor and bar-code scanner</td>
</tr>
</tbody>
</table>

### Kits and Reagents for the Isolation of DNA

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Cat. No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure Compact Nucleic Acid Isolation Kit I+</td>
<td>Genomic DNA from mammalian whole blood or cultured cells</td>
<td>03 730 964 001</td>
<td>1 kit (32 isolations) including all required plastic disposables</td>
</tr>
<tr>
<td></td>
<td>Viral nucleic acids from plasma or serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sample volume range 100 μl – 400 μl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MagNA Pure Compact Nucleic Acid Isolation Kit I+ – Large Volume</td>
<td>Genomic DNA from mammalian whole blood or cultured cells</td>
<td>03 730 972 001</td>
<td>1 kit (32 isolations) including all required plastic disposables</td>
</tr>
<tr>
<td></td>
<td>Viral nucleic acids from plasma or serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sample volume range 500 μl – 1000 μl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MagNA Pure Bacteria Lysis Buffer+</td>
<td>DNA from bacteria in many different sample types, such as urine, BAL (bronchoalveolar lavage), sputum, CSF, swabs, or bacterial cultures</td>
<td>04 659 180 001</td>
<td>20 ml</td>
</tr>
<tr>
<td>MagNA Pure Tissue Lysis Buffer+</td>
<td>Genomic DNA from mammalian tissue. Sample volume range 1 – 10 mg</td>
<td>04 805 160 001</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

### Kit for the Isolation of RNA

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Cat. No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure Compact RNA Isolation Kit+</td>
<td>RNA from mammalian tissue, blood, cultured cells, and blood cells</td>
<td>04 802 993 001</td>
<td>1 kit (32 isolations) including all required plastic disposables</td>
</tr>
<tr>
<td></td>
<td>Sample amount up to 10 mg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* For general laboratory use.

For detailed information about the MagNA Pure LC System and accessories, please visit www.magnapure.com
## Companion Reagents for Isolating Nucleic Acids

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteinase K* recombinant PCR Grade, solution</td>
<td>Non-specific enzyme for digesting cellular proteins</td>
<td>Rapid inactivation of endogenous nucleases and isolation of nucleic acid from tissues or cell lines</td>
</tr>
<tr>
<td>Proteinase K* recombinant PCR Grade, lyophilizate</td>
<td>See above</td>
<td>See above</td>
</tr>
<tr>
<td>Pronase* from <em>Streptomyces griseus</em></td>
<td>Pronase is a various mixture of proteases with different proteolytic activities</td>
<td>In conjunction with other enzymes (e.g. collagenase, trypsin), pronase is suitable for the isolation of a variety of cell types</td>
</tr>
<tr>
<td><strong>Nucleases and nuclease inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNase I recombinant, RNase-free* from bovine pancreas, expressed in <em>Pichia pastoris</em></td>
<td>Endonuclease for double- and single-stranded DNA, free of ribonuclease and protease</td>
<td>Isolation of DNA-free RNA</td>
</tr>
<tr>
<td>RNase, DNase-free*</td>
<td>RNase mixture, free of contaminating DNases</td>
<td>Isolation of RNA-free DNA</td>
</tr>
<tr>
<td>RNase A*</td>
<td>Pyrimidine-specific endonuclease that acts on single-stranded RNA</td>
<td>Isolation of genomic DNA</td>
</tr>
<tr>
<td>RNase H*</td>
<td>Endonuclease that cleaves RNA in RNA:DNA hybrids</td>
<td>Elimination of RNA template after first strand cDNA synthesis</td>
</tr>
<tr>
<td>Protector RNase Inhibitor*</td>
<td>Recombinant from rat lung</td>
<td>Protects mRNA and total RNA during isolation and cDNA synthesis</td>
</tr>
<tr>
<td><strong>Additional reagents required in some purification procedures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agarose LE* low electro endosmosis</td>
<td>Electrophoretic gel medium</td>
<td>Electrophoretic separation of nucleic acids, size range 0.2 – 15 kbp</td>
</tr>
<tr>
<td>Agarose MS* molecular screening</td>
<td>Electrophoretic gel medium</td>
<td>Electrophoretic separation of nucleic acids, of PCR products genotyping allele sizing, STR analysis</td>
</tr>
<tr>
<td>Agarose MP* multi purpose agarose</td>
<td>Electrophoretic gel medium</td>
<td>Electrophoretic separation of nucleic acids, especially high molecular weight DNA</td>
</tr>
<tr>
<td>Cesium chloride* (CsCl), MB grade</td>
<td>Standard centrifugation medium</td>
<td>Separation and purification of nucleic acids by density</td>
</tr>
<tr>
<td>Glycogen* solution</td>
<td>Inert macromolecule</td>
<td>Carrier for the precipitation of nucleic acids</td>
</tr>
<tr>
<td>Guanidine thiocyanate*</td>
<td>Crystalline denaturant</td>
<td>Protein denaturing agent; component of buffers</td>
</tr>
<tr>
<td>Nonidet P40*</td>
<td>Non-ionic detergent</td>
<td>Component of buffers; stabilizes enzymes</td>
</tr>
<tr>
<td>Poly(A)*</td>
<td>Polyadenylic acid, sodium salt</td>
<td>RNA carrier for precipitation</td>
</tr>
</tbody>
</table>

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### Features

<table>
<thead>
<tr>
<th>Features</th>
<th>Cat. No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ideal for isolation of nucleic acids, since it is virtually free of RNases and DNases</td>
<td>03 115 887 001</td>
<td>1.25 ml</td>
</tr>
<tr>
<td></td>
<td>03 115 828 001</td>
<td>5 ml</td>
</tr>
<tr>
<td>Enhances isolation of intact nucleic acids, since it rapidly inactivates endogenous nucleases</td>
<td>03 115 844 001</td>
<td>25 ml</td>
</tr>
<tr>
<td>Especially suited for isolation of PCR templates, since preparation has minimized level of contaminants.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Same as Proteinase K solution</td>
<td>03 115 836 001</td>
<td>25 mg</td>
</tr>
<tr>
<td></td>
<td>03 115 879 001</td>
<td>100 mg</td>
</tr>
<tr>
<td></td>
<td>03 115 801 001</td>
<td>2 x 250 mg</td>
</tr>
<tr>
<td></td>
<td>03 115 852 001</td>
<td>4 x 250 mg</td>
</tr>
<tr>
<td>Total degradation of proteins during the isolation of DNA and RNA (it is not necessary to let pronase self-digest prior to use)</td>
<td>10 165 921 001</td>
<td>1 g (non-sterile)</td>
</tr>
<tr>
<td></td>
<td>11 459 643 001</td>
<td>5 g (non-sterile)</td>
</tr>
<tr>
<td>Ideal for the isolation of RNA, since it is virtually free of RNases</td>
<td>04 716 728 001</td>
<td>10,000 U</td>
</tr>
<tr>
<td>Rely on a recombinant animal-free enzyme</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ideal for isolation of DNA, since it is virtually free of DNases</td>
<td>11 119 915 001</td>
<td>500 μg</td>
</tr>
<tr>
<td>Unlike most RNase preparations, does not need to be boiled prior to use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Used in many DNA isolation procedures</td>
<td>10 109 142 001</td>
<td>25 mg</td>
</tr>
<tr>
<td></td>
<td>10 109 169 001</td>
<td>100 mg</td>
</tr>
<tr>
<td>Eliminates potential source of PCR inhibition</td>
<td>10 786 349 001</td>
<td>25 units</td>
</tr>
<tr>
<td></td>
<td>10 786 357 001</td>
<td>100 units</td>
</tr>
<tr>
<td>Active over a broad pH range (pH 5–8)</td>
<td>03 335 399 001</td>
<td>2000 units</td>
</tr>
<tr>
<td>Virtually free of endonucleases and DNA nicking activities</td>
<td>03 335 402 001</td>
<td>10,000 units</td>
</tr>
<tr>
<td>May be used in one-step or two-step RT-PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Used in many DNA isolation procedures</td>
<td>10 109 134 001</td>
<td>500 mg</td>
</tr>
<tr>
<td>Can be used for the analysis of PCR product, examination of restriction enzyme digest of plasmid, cosmid and λ-phage DNA and electrophoresis of RNA</td>
<td>11 685 660 001</td>
<td>100 g</td>
</tr>
<tr>
<td></td>
<td>11 685 678 001</td>
<td>500 g</td>
</tr>
<tr>
<td>Resolve PCR fragments (50 bp – 1500 bp)</td>
<td>11 816 586 001</td>
<td>100 g</td>
</tr>
<tr>
<td>Virtually free of DNases and RNases</td>
<td>11 816 594 001</td>
<td>500 g</td>
</tr>
<tr>
<td>Discriminate between fragments that differ in only 4 bp of length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suitable for analytical and preparative isolation</td>
<td>11 388 983 001</td>
<td>100 g</td>
</tr>
<tr>
<td>Virtually free of DNases and RNases</td>
<td>11 388 991 001</td>
<td>500 g</td>
</tr>
<tr>
<td>Separation of high molecular weight DNA (PFGE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very highly purified preparation</td>
<td>10 757 306 001</td>
<td>1 kg</td>
</tr>
<tr>
<td>Prepared especially for molecular biology procedures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unlike tRNA, will not interfere with downstream procedures</td>
<td>10 901 393 001</td>
<td>20 mg</td>
</tr>
<tr>
<td>Very low absorbance at 260 and 280 nm, so it doesn’t interfere with UV detection of nucleic acids</td>
<td>11 685 929 001</td>
<td>500 g</td>
</tr>
<tr>
<td>Stronger protein denaturant than guanidine hydrochloride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highly purified</td>
<td>11 332 473 001</td>
<td>5 x 10 ml</td>
</tr>
<tr>
<td>Virtually free of enzyme inhibitors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Readily soluble salt</td>
<td>10 108 626 001</td>
<td>100 mg</td>
</tr>
</tbody>
</table>
Companion Reagents for Isolating Nucleic Acids, continued

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Additional reagents required in some purification procedures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium Dodecyl Sulfate (SDS), electrophoresis grade*</td>
<td>Anionic detergent; highly purified</td>
<td>Denaturation of proteins before or during electrophoresis; dissociation of protein-nucleic acid complexes</td>
</tr>
<tr>
<td>Tris*</td>
<td>Biological buffer, crystalline</td>
<td>Preparation of buffers, pH 7-9, for biological and biochemical applications</td>
</tr>
<tr>
<td>Triton X-100*</td>
<td>Non-ionic detergent</td>
<td>Solubilization agent for proteins</td>
</tr>
</tbody>
</table>
## Features

<table>
<thead>
<tr>
<th>Features</th>
<th>Cat. No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapidly and completely denatures most proteins</td>
<td>11 667 289 001</td>
<td>1 kg</td>
</tr>
<tr>
<td>Particularly suited for gel electrophoresis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virtually free of proteases, RNases, and DNases</td>
<td>10 708 976 001</td>
<td>1 kg</td>
</tr>
<tr>
<td>pK 8.3 (at +20°C), which makes Tris ideal for preparing buffers in the biological pH range</td>
<td>11 814 273 001</td>
<td>5 kg</td>
</tr>
<tr>
<td>Used in many published purification procedures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrapure</td>
<td>11 332 481 001</td>
<td>5 x 10 ml</td>
</tr>
</tbody>
</table>

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CSPD is a trademark of Applera Corporation.

RNAlater is a registered trademark of Ambion, Inc.

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### Disclaimer

* For life science research only. Not for use in diagnostic procedures.

† For general laboratory use.

‡ CE/for USA for laboratory use
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<th>Agarose MP</th>
<th>0.1 kbp - 30 kbp (Standard size range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose LE</td>
<td>0.2 kbp - 15 kbp</td>
</tr>
<tr>
<td>Agarose MS</td>
<td>0.05 kbp - 1.5 kbp</td>
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