MagNA Pure LC DNA Isolation Kit II (Tissue) – a New Tool for Automated DNA Isolation

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Introduction

To broaden the possibilities of the MagNA Pure LC system, a new kit for the isolation of DNA from various types of tissue was developed. The protocol contains only one manual step, the mechanical disruption and lysis of the tissue sample using well-established homogenization methods like rotor-stator, bead-mill, or mortar/pestle, and a special tissue lysis buffer. All subsequent DNA isolation steps are performed automatically by the MagNA Pure LC based on the magnetic bead technology. First, cells are lysed and proteins digested by incubation with proteinase K. Then a special lysis/binding buffer and magnetic glass particles (MGP) are added. The DNA binds to the MGP surface and is purified by several washing steps. Finally the DNA is eluted in a special buffer at elevated temperature. The kit was tested with a variety of tissue types. Tests included criteria like DNA integrity, yield, purity, reproducibility, scalability, cross contamination, and comparisons to other methods.

Materials and Methods

Homogenization of tissue

For one isolation, 1 – 10 mg of mouse tissue (liver, kidney, spleen, tail, brain, ear, muscle, lung) were homogenized using various methods according to the instrument supplier's instructions (e.g., FastPrep/Ribolyser: 10 – 60 seconds at V_{max} in disposable tubes) with addition of tissue lysis buffer from the kit. In most cases, multiples of these amounts were processed to compensate the dead volume of the respective homogenization method and to allow analyses in replicates. For some tissues (e.g., mouse tail) up to 25 mg tissue per isolation were tried.

Table 1: Yield, purity and LightCycler PCR results of DNA isolated from various mouse tissues

Tissue type	Amount [mg]	DNA yield [µg]	Purity [OD_{260}/OD_{280}]
Liver	10	18	1.7
Kidney	10	18	1.8
Tail	10	10	1.7
Spleen	10	40	1.9
Brain	10	22	1.7
Ear	10	16	1.7
Muscle	10	4	1.7
Lung	10	25	1.8

Optional RNase digestion step

Some tissues contain large amounts of RNA. To eliminate RNA co-isolated from these tissues, an RNase A solution was added to the lysate and incubated for 15 minutes at 65 °C. This allows a more reliable photometric quantification of the purified DNA.

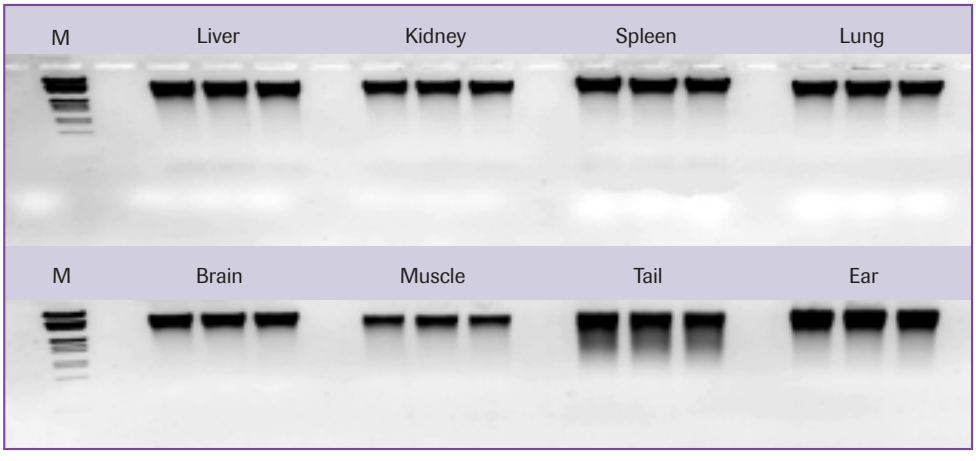


Figure 1:
DNA integrity: DNA was isolated from various types of mouse tissue and analyzed on a 1 % agarose gel. All samples were done in triplicates

DNA isolation on the MagNA Pure LC

The lysate was then transferred to the sample cartridge of the MagNA Pure LC, loaded onto the workstation together with the necessary disposables and kit reagents, and the automated DNA isolation was started. The MagNA Pure LC automatically performs all isolation and purification steps like proteinase K digest, binding of DNA, washing steps, elution of the purified DNA, and transfer to a cooled storage cartridge.

Analysis of the isolated DNA

The integrity of the isolated DNA was checked on a 1 % agarose gel together with molecular weight marker III (Roche Molecular Biochemicals). The DNA yields were calculated from the $OD_{260\,nm}$ measurement, the purity was assessed by calculating the ratio $OD_{260\,nm}/OD_{280\,nm}$. To check the amplificability of the DNA, a LightCycler PCR was performed for all samples using cyclophilin A specific primers and Hybridization Probes.

Results and Discussion

DNA integrity

Agarose gel analysis of the DNA from various tissues showed, that the DNA was of high integrity (Figure 1). The molecular weight range of the isolated DNA was usually about 10 - 20 kb, and was largely dependent on the intensity of the mechanical tissue homogenization.

Yield and purity

OD analysis revealed yields of up to 40 µg genomic DNA from 10 mg tissue (Table 1). Yields were similar to or higher than e.g. filter tube methods. The $OD_{260\,nm}/OD_{280\,nm}$ ratio was 1.8 +/- 0.1, indicating DNA of high purity.

Reproducibility

Isolation of DNA from various tissues in 6-fold to 32-fold replicates showed excellent reproducibility. The coefficient of variation (CV) for yield and purity was <10 %, with regard to LightCycler Crossing Points the CV was <3 % (Figure 2).

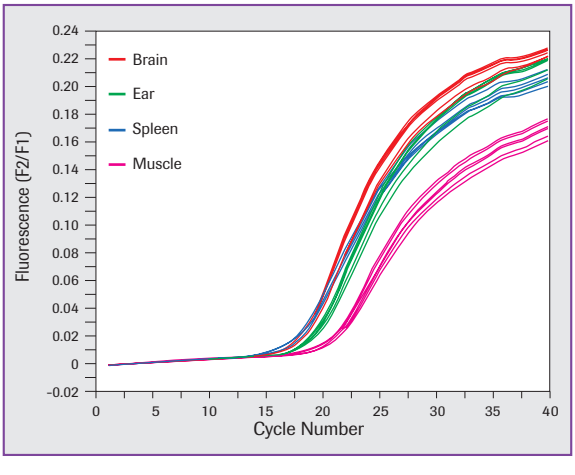


Figure 2: LightCycler PCR with DNA isolated from different mouse tissues. All samples were analyzed in 6-fold replicates

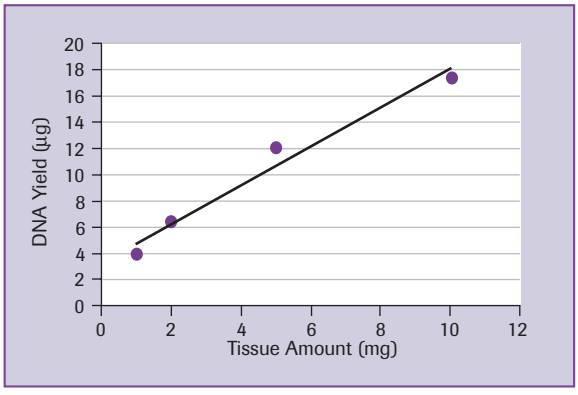


Figure 3: Scalability of yield ($OD_{260\,nm}$). DNA was isolated from 1, 2, 5 and 10 mg mouse liver, and the yield determined by $OD_{260\,nm}$

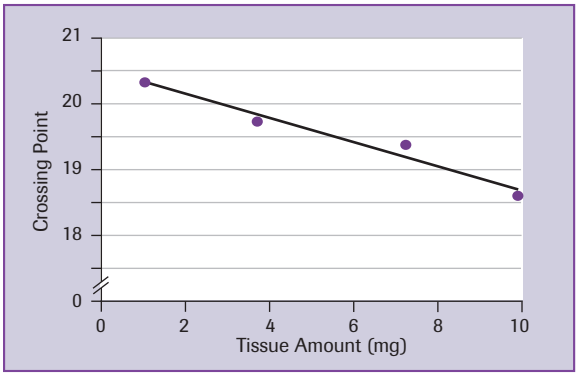


Figure 4: Scalability of yield (LightCycler PCR). DNA was isolated from 1, 2, 5 and 10 mg mouse liver, and analyzed by quantitative LightCycler PCR. The Crossing Points reflect the amount of input DNA

Scalability

DNA was isolated from varying amounts of mouse tissue (1 - 10 mg). Analysis by gel, OD and LightCycler showed a good scalability (Figures 3 and 4). For some tissues (liver, kidney, tail, muscle, ear) up to 25 mg starting material was used and could be processed with good results.

Cross contamination

Every second well of the MagNA Pure LC sample cartridge was filled with tissue lysate from 5 mg mouse liver, the neighboring wells with buffer, respectively (checker board pattern). After MagNA Pure LC DNA isolation and subsequent LightCycler PCR analysis (45 cycles), no cross contamination was found in the 16 buffer samples, while the 16 tissue samples showed the expected signal (data not shown).

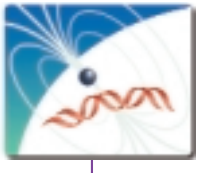
Summary

The MagNA Pure LC DNA Isolation Kit II (Tissue) proved to be a valuable tool for efficient, automated isolation of DNA from various tissues. The only manual step is the tissue homogenization, which can be done efficiently using semi-automated methods like FastPrep, Ribolyser etc. (about 15 - 30 minutes for 32 samples). All other steps including the proteinase K incubation are performed automatically by the MagNA Pure LC System within 43 - 105 minutes, depending on the number of samples. The isolated DNA is of high quality and shows no PCR inhibition. The reproducibility is excellent and no cross contamination is found. Thus the new MagNA Pure LC DNA Isolation Kit II (Tissue) allows new applications of the MagNA Pure LC/LightCycler System and further simplifies nucleic acid analysis.

http://biochem.roche.com/magnapure

Order INFO

Product	Pack Size	Cat. No.
MagNA Pure LC DNA Isolation Kit II (Tissue)	1 kit (192 isolations)	3 186 229
MagNA Pure LC Instrument	1 instrument plus accessories	2 236 931



Process Prelysed Samples and Use Variable Elution Volumes when Isolating Nucleic Acids with the MagNA Pure LC

To extend further the possibilities of the MagNA Pure LC system three new protocols running on the MagNA Pure LC Software are now available

MagNA Pure LC Total Nucleic Acid Isolation Protocol for Variable Elution Volume:

This protocol is optimized to run with the MagNA Pure LC Total Nucleic Acid Isolation Kit and offers the possibility to use variable elution volumes in a range of 50 - 100 µl for the isolation of viral nucleic acids (DNA and RNA) from 50 - 200 µl serum and plasma samples. Thus the concentration of total nucleic acid in the eluates can be flexibly increased.

MagNA Pure LC Total Nucleic Acid Isolation Protocol for External Lysis:

By offering the possibility to load externally prelysed samples such as serum or plasma on the MagNA Pure LC, this protocol brings additional flexibility to the MagNA Pure LC. Again it is optimized to run with the MagNA Pure LC Total Nucleic Acid Isolation Kit for the isolation of viral nucleic acids (DNA and RNA) from 50 - 200 µl serum or plasma samples. The protocol also includes the above mentioned variable elution volumes.

MagNA Pure LC mRNA Isolation Kit I Protocol for External Blood Lysis:

Similar as described above this protocol offers the possibility to load prelysed blood samples on the MagNA Pure LC. This protocol, however, is optimized to run with the MagNA Pure LC mRNA Isolation Kit I isolating highest quality mRNA from blood samples with up to 100 µl sample volume.

All protocols are delivered free of charge on a floppy disk. A detailed installation guide is included. **To order please contact your local Roche representative.**

Order INFO

Product	Pack Size	Cat. No.
MagNA Pure LC Total Nucleic Acid Isolation Kit	1 kit (192 reactions)	3 038 505
MagNA Pure LC mRNA Isolation Kit I	1 kit (192 reactions)	3 004 015
MagNA Pure LC Total Nucleic Acid Isolation - Protocol for External Lysis	1 floppy disk	3 188 892
MagNA Pure LC Total Nucleic Acid Isolation - Protocol for Variable Elution Volume	1 floppy disk	3 142 680
MagNA Pure LC mRNA Isolation Kit I - Protocol for External Lysis	1 floppy disk	3 189 520

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