MagNA Pure Compact RNA Isolation Kit: Isolation of High-Quality Total RNA from a Broad Range of Sample Material

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Introduction

The MagNA Pure Compact Instrument together with the new MagNA Pure Compact RNA Isolation Kit can be used for automated isolation of total RNA from blood, blood cells, tissue, and cultured cells. The MagNA Pure Compact System perfectly fits into Roche Applied Science’s integrated solution for real-time PCR: The MagNA Lyser Instrument, Universal ProbeLibrary, Transcriptor First Strand cDNA Synthesis Kit and FastStart TaqMan® Probe Master. The basic procedure of the RNA isolation is based on the MagNA Pure Magnetic Glass Particle (MGP) Technology. The principle steps include lysis of samples in a special tissue lysis buffer containing chaotropic salt and protease K that destroys remaining proteins including nucleases. Nucleic acids are immobilized on the MGP surfaces and genomic DNA is degraded by incubation with DNase. Unbound substances are removed by several washing steps and finally, purified RNA is eluted from the MGPs. The aim of this work was to assess the efficacy of the MagNA Pure Compact RNA Isolation Kit to extract total RNA from different tissues and cell lines. In addition to the assessment of purity and integrity, the suitability of extracted RNA to down-stream applications such as real-time PCR and microarray-based gene expression analysis was tested.

Materials and Methods

Tissue and cell samples

SK-N-DZ cells (human neuroblastoma; ATCC no. CRL-2149) were cultured in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and 4 mM L-glutamine. PC 12 cells (rat pheochromocytoma; ATCC no. CRL-1721) were cultured in Ham’s F12K medium supplemented with 15% horse serum, 2.5% fetal bovine serum and 2 mM L-glutamine. Human tissue samples were obtained from the Department of Pathology at the University Hospital of North Norway, Tromsø. Animal tissue samples were obtained from Sprague-Dawley rats. Animals were maintained in our animal facilities on standard laboratory chow.

Isolation of RNA

Tissue specimens were either used directly, fresh-frozen, or stored in RNA Later Solution. Disruption and homogenization of tissue samples were performed using the MagNA Lyser Instrument, according to the instructions. Cell culture samples were directly homogenized in PBS and lysis buffer included in the kit. The elution volume was set to 50 µl.

Quantity and purity of total RNA

RNA was quantified measuring absorbance at 260 nm and RNA purity was determined by the ratios OD_{260 nm}/280 nm and OD_{260 nm}/230 nm using the NanoDrop instrument. The RNA integrity (RIN) was determined by electrophoresis using the Agilent Bioanalyzer 2100.

Quantification of mRNA by real-time RT-PCR of cyclophilin A

Three microliters of the eluted RNA were reversely transcribed with Transcriptor First Strand cDNA Synthesis Kit. The real-time PCR assay targeting cyclophilin A was performed with 250 ng cDNA using a real-time PCR instrument, FastStart TaqMan® Probe Master, and...
Table 1: RNA yield and purity. RNA was isolated from tissue or cultured cell samples. Elution volume was set to 50 μl, and RNA yield and purity were determined by OD_{260 nm/280 nm} and OD_{260 nm/230 nm} measurements. The table shows typical ranges of RNA yields and average OD_{260 nm/280 nm} and OD_{260 nm/230 nm} ratios for up to ten independent preparations each.

<table>
<thead>
<tr>
<th>Sample Material</th>
<th>Amount</th>
<th>Average Yield</th>
<th>Average OD_{260 nm/280 nm} Ratio</th>
<th>Average OD_{260 nm/230 nm} Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Colon</td>
<td>10 mg</td>
<td>14 – 32 μg</td>
<td>1.97 – 2.07</td>
<td>2.04 – 2.14</td>
</tr>
<tr>
<td>Rat Lung</td>
<td>10 mg</td>
<td>7 – 11 μg</td>
<td>2.07 – 2.08</td>
<td>2.07 – 2.08</td>
</tr>
<tr>
<td>Rat Liver</td>
<td>10 mg</td>
<td>27 – 36 μg</td>
<td>1.94 – 2.07</td>
<td>2.05 – 2.12</td>
</tr>
<tr>
<td>Rat Brain</td>
<td>10 mg</td>
<td>3 – 6 μg</td>
<td>2.05 – 2.10</td>
<td>1.52 – 1.93</td>
</tr>
<tr>
<td>SK-N-DZ (human)</td>
<td>5 x 10^5 cells</td>
<td>14 – 17 μg</td>
<td>2.05 – 2.06</td>
<td>2.04 – 2.06</td>
</tr>
<tr>
<td>PC 12 (rat)</td>
<td>5 x 10^5 cells</td>
<td>17 – 21 μg</td>
<td>2.03 – 2.05</td>
<td>2.03 – 2.07</td>
</tr>
</tbody>
</table>

Universal ProbeLibrary probes (human probe #48 and rat probe #42). As a negative control, RNA was replaced by water. To prove the absence of residual genomic DNA within RNA extractions, RNA samples were directly subjected to PCR targeting cyclophilin A using 2 ng of human genomic DNA as positive control.

Microarray experiments
In three independent dye-flip experiments, 3 μg of total RNA from rat liver and rat lung were labeled with Cy5 and/or Cy3 using the 3 DNA Array 350 HS Kit. Hybridizations were performed with a TECAN 400HS instrument. Rat oligo arrays were purchased from the Norwegian Microarray Consortium (NMC). Experiments were performed at the Microarray Resource Centre Tromso (MRCT). The arrays were normalized using three dye-flip replicates. This reduced the set of six arrays into three normalized versions.

M-M (log-ratio versus log-ratio) scatter plots of the dye-normalized replicates were then produced. The correlation coefficients of the replicates were finally calculated using the Pearson correlation function.

Results and Applications
To measure the quality and amount of total RNA extracted using the MagNA Pure Compact Instrument and the MagNA Pure Compact RNA Isolation Kit, we measured the absorbance at 230 nm, 260 nm and 280 nm, conducted LabChip (Agilent) microfluidic analysis and performed quantitative real-time PCR of cyclophilin A.

In addition, we tested the suitability of the extracted total RNA for microarray analysis. We demonstrated that total RNA preparations from different human and rat tissues and from cell lines isolated with the MagNA Pure Compact

![Amplification plot](image)

Figure 2: Example of a real-time PCR analysis (targeting cyclophilin A). (a) Eight RNA samples isolated from human colon and (b) a minus-RT-PCR of eight RNA preparations of each tissue and cell sample as shown in Table 1, with human genomic DNA as positive control.
Instrument yield excellent RNA in sufficient amounts. Electrophoresis and the ratios of OD_{260 nm}/OD_{280 nm} show that high quality total RNA was extracted (Figure 1, Table 1). The integrity (RIN) of RNA samples was in a very good range with respect to the different tissues tested. The lower OD_{260 nm}/OD_{230 nm} ratio of isolations from rat brain is most likely due to the relatively high lipid and fatty acid composition in brain tissue.

The reproducibility of RNA extraction was evaluated by real-time PCR for RNA extracted from human colon. The low CVs of crossing points calculated by quantification analysis of the amplification curves showed good reproducibility of the isolation procedure (Figure 2a). All preparations were virtually free of residual genomic DNA (Figure 2b) and no signs of PCR inhibition were observed (Figure 2a). In addition, dye-flip microarray experiments with RNA from rat liver versus rat lung displayed high log-ratio correlations between replicates, indicating that the extracted RNA is suitable for differential expression studies (Figure 3). The correlation coefficients were calculated using all available features on the arrays. Therefore, the values indicate a high degree of consistency between replicates.

Conclusions

In conclusion, the MagNA Pure Compact Instrument and MagNA Pure Compact RNA Isolation Kit facilitate the preparation of highly purified total RNA from fresh and fresh-frozen animal tissue as well as cultured cell samples. Tissue samples stabilized by specific reagents (e.g., RNA Later) can also be used. The total time for automated purification of RNA from eight samples is approximately 35 minutes. The purified RNA from tissues and cells is an ideal starting material for down-stream applications such as real-time PCR and microarray-based gene expression analysis.