Evaluation of the MagNA Pure LC mRNA Isolation Kit II (Tissue)

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Introduction

Isolation of pure, intact mRNA in large amounts is of great importance and the fundamental key for monitoring tissue-specific gene expression. MagNA Pure LC mRNA Isolation Kit II (Tissue) is designed to purify mRNA from 1 - 10 mg frozen animal or human tissue on the MagNA Pure LC Instrument. The kit contains all buffers (Table 1) required to perform 192 (6 x 32) mRNA isolations, no additional reagents are necessary. The mRNA isolation procedure is based on magnetic-bead technology with reagents inhibiting nucleases at the earliest purification steps. These features together with the automated processing enable one to obtain DNA-free mRNA with high yield and quality as is required for all downstream applications regarding gene expression analysis.

Table 1: MagNA Pure LC mRNA Isolation Kit II (Tissue) – Content and function

Reagent	Function
Bi-Oligo(dT) probe, biotin labeld	For hybridization of Bi-Oligo(dT) to mRNA
Hybridization Buffer	For dilution of Bi-Oligo(dT)
Wash Buffer II	For removing salts, proteins ect.
Lysis Buffer	For cell lysis and inactivation of nucleases
DNase, RNase-free	For DNA digestion
DNase Incubation Buffer	For dilution of DNase
Wash Buffer I	For removing PCR inhibitors
Streptavidin Magnetic Particles (SMPs) complex	For binding of Bi-Oligo(dT)/mRNA
Elution Buffer	For elution of pure mRNA

The principle of mRNA isolation with the MagNA Pure LC mRNA Isolation Kit II (Tissue) is based on the use of biotin-labeled oligo(dT) and streptavidin-coated magnetic particles (SMPs) (Figure 1). First, a manual pre-isolation step is performed (sample lysis and homogenization). The latter steps of the purification procedure have been optimized for the performance of the MagNA Pure LC Instrument. A specific protocol for mRNA isolation from tissue installed in the MagNA Pure LC Instrument software is used. The software automatically calculates the necessary amounts of all reagents, indicates the amount and the type of disposable required and guides the user through the instrument set up.

The first results obtained with the MagNA Pure LC mRNA Isolation Kit II (Tissue) are shown here. mRNA was isolated from different types and amounts of frozen mouse tissue (1 - 10 mg spleen, liver and kidney). The mRNA quality was checked by RT-PCR analysis and absence of DNA contamination was determined by applying a minus RT control PCR. The integrity and purity of the mRNA was evaluated by Northern blot analysis. The reproducibility of the MagNA Pure LC system was checked by RT-PCR analysis on the LightCycler PCR system.

Materials and Methods

mRNA isolation from frozen mouse tissue on the MagNA Pure LC Work Station

➡ Pre-isolation steps

Homogenization of 1 - 10 mg frozen (– 80 °C) mouse liver, spleen and kidney was carried out using the Lysis Buffer and the homogenization instrument (Ribolyser [Hybaid], UltraTurrax [IKA] or mortar/pestle/ needle). The Capture Buffer containing biotin-labeled Oligo(dT) was added. The released poly(A^+)-tailed mRNA hybridized specifically to the oligo(dT). Following centrifugation, the lysate supernatant was recovered and transferred into the Magna Pure LC Sample Cartridge.

➡ Automated mRNA isolation

The mRNA/Oligo(dT) complex was immobilized onto the Streptavidin Magnetic Particles (SMPs) surface. The bead complex was incubated with DNase to eliminate DNA contamination. The SMPs were washed several times to remove any unbound substances. Finally, the mRNA was eluted from the SMPs and recovered in a final volume of 100 µl.

LightCycler RT-PCR and minus RT control PCR analysis

After nucleic acid isolation, the MagNA Pure LC Instrument sets up the RT-PCR and minus RT control PCR. Both

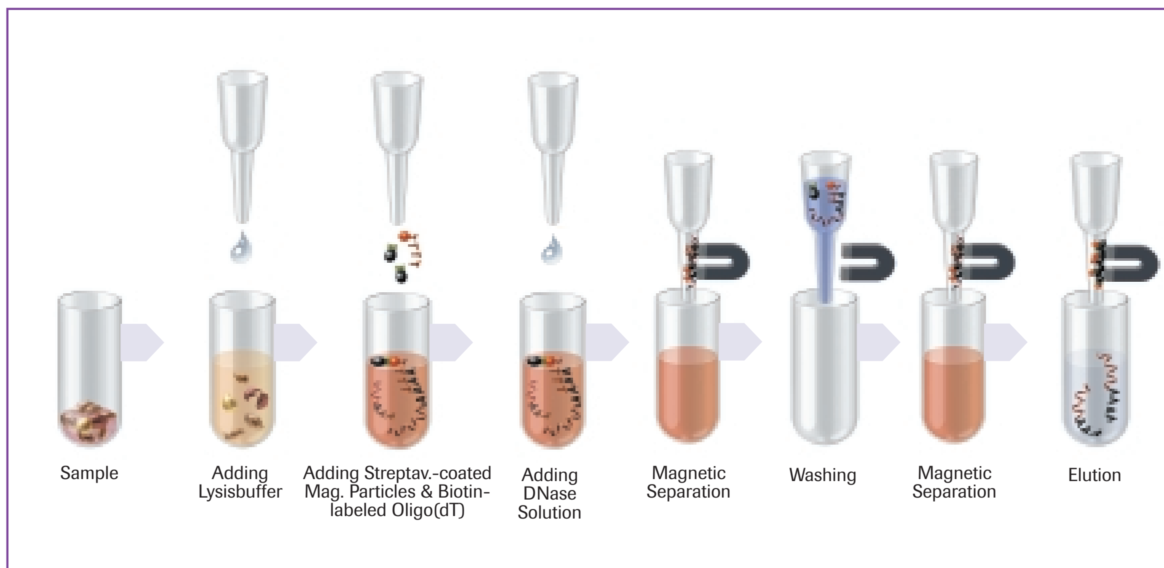


Figure 1: Principle of mRNA isolation from frozen animal or human tissue performed automatically by the MagNA Pure LC Instrument

MAGNA PURE LC

LightCycler RT-PCR and minus RT control PCR were performed in 20 µl final volume reaction using 5 µl of isolated mRNA sample and the Hybridization Probe format.

Northern blot analysis

Isolated mRNA was used for Northern blot analysis [1]. 7.5 µl of the 100 µl mRNA eluate were separated by denaturing gel electrophoresis, transferred onto a Nylon membrane and hybridized overnight at 68 °C with the β-actin transcript digoxigenin(DIG)-labeled probe. The results were monitored by the Lumi-Imager F1 Workstation.

Figure 2: Northern blot analysis of the β-actin expression on mRNA isolated from 10 mg frozen mouse liver, kidney and spleen. 7.5 µl out of the 100 µl mRNA eluate from mouse liver (lanes 1 and 2) kidney, (lanes 3 and 4) and spleen (lanes 5 and 6) were in duplicate separated by denaturing agarose gel electrophoresis, transferred onto a Nylon membrane and hybridized with the β-actin DIG-labeled probe. mRNA was isolated either using the Ribolyser (A) or Ultra Turrax (B) or mortar/pestle (C) as homogenization method

Results

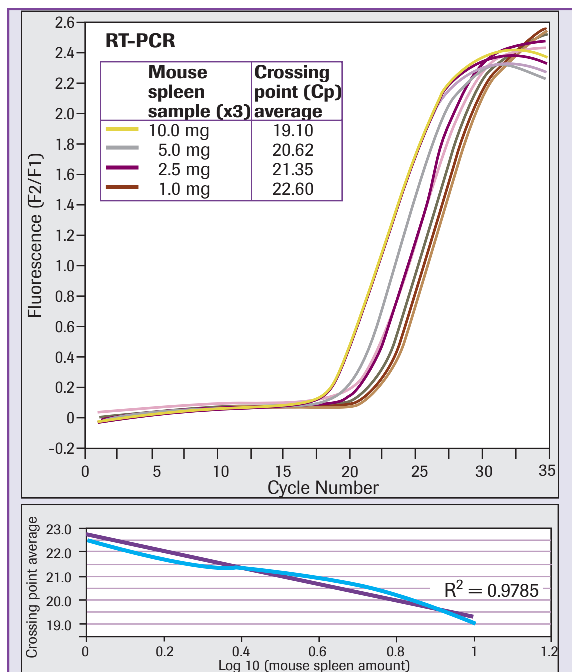
Northern blot analysis

The integrity of mRNA isolated with the MagNA Pure LC mRNA Isolation Kit II (Tissue) was confirmed by Northern blot analysis using the DIG-labeled β-actin transcript as probe. mRNA was isolated from 10 mg of different tissue types (mouse liver, spleen and kidney) using 3 different homogenization methods (Ribolyser, Ultra Turrax and mortar/pestle). The picture of the hybridization pattern showed one distinct band of the expected size (1.8 kb) for each sample (Figure 2). The mRNA samples isolated using the Ribolyser as homogenization method showed a stronger band than other methods. These results demonstrate that the homogenization with the Ribolyser leads to the highest mRNA yield with respect to other methods tested.

The Northern blot analysis clearly showed that mRNA isolated with the MagNA Pure LC mRNA Isolation Kit II (Tissue) was not degraded and of high integrity even when applying material containing large amounts of endonucleases such as liver. This demonstrates the particular efficiency of the MagNA Pure LC mRNA Isolation Kit II (Tissue) reagents in inhibiting nucleases very early in the purification process.

Scalability analysis

Performance of the MagNA Pure LC system in reproducing the scale of the starting material amounts was checked by isolating mRNA from different amounts of mouse tissue (spleen and liver) using the Ribolyser as homogenization method. Isolated mRNA was used to amplify the cyclophilin-A transcript in a one step RT-PCR with the LightCycler Instrument. The results of the RT-PCR



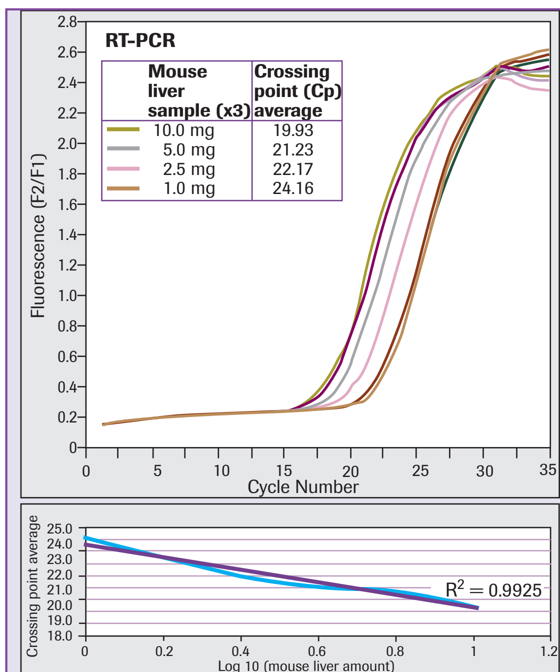


Figure 3: RT-PCR analysis of mRNA isolated from different amounts of mouse spleen.
5 µl mRNA eluate was used as template for amplification of the cyclophilin-A transcript by the LightCycler using the Hybridization Probe format. The crossing points (Cp) average values of the RT-PCR run were calculated from three repetitions and a graphic was deduced where regression coefficients (R) were calculated

Figure 4: RT-PCR analysis of mRNA isolated from different amount of mouse liver.
5 µl mRNA eluate was used as template for amplification of the cyclophilin-A transcript by the LightCycler using the Hybridization Probe format. The crossing points (Cp) average values of the RT-PCR run were calculated from three repetitions and a graphic was deduced where regression coefficients were calculated

are presented in Figures 3 and 4. With both tissue types RT-PCR graphs show a regular shift and the analysis of the crossing point values shows a linear pattern with a regression coefficient of 0.97 and 0.99, respectively. Both results reflect the difference in the starting material amounts.

These results demonstrate that the amount of nucleic acid isolated with the MagNA Pure LC mRNA Isolation Kit II (Tissue) together with the instrument strictly corresponds to the amount of starting material. This is one of the fundamental requirements for studying gene expression.

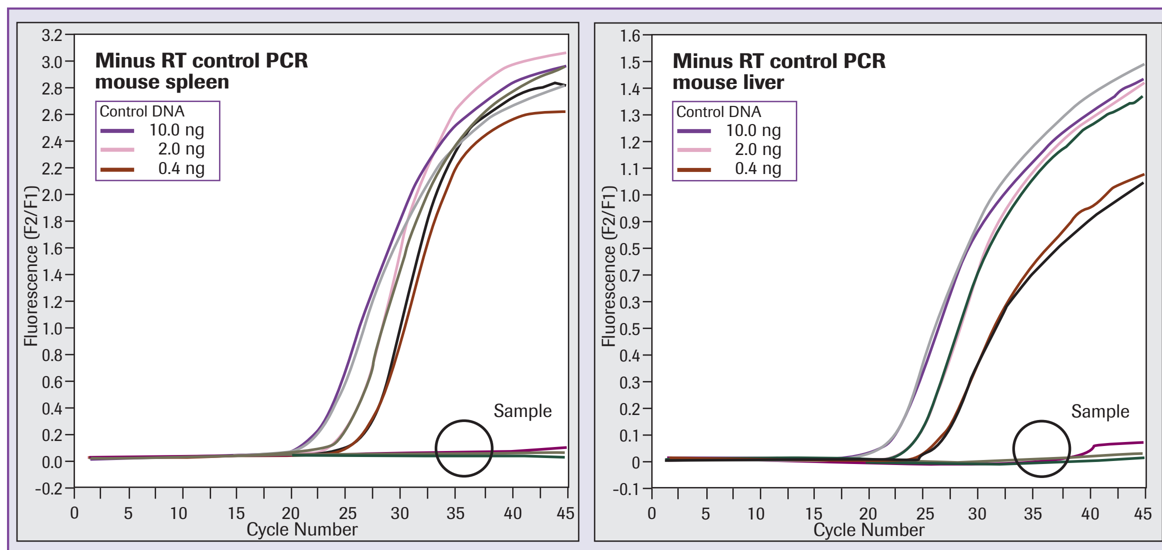


Figure 5: Minus RT control PCR analysis of mRNA isolated from different amounts of mouse spleen and liver.
5 µl mRNA eluate from 10, 5, 2.5 and 1 mg mouse spleen and liver were used as templates for amplification of the cyclophilin-A gene by the LightCycler using the Hybridization Probe format. Control of 10, 2 and 0.4 ng human genomic DNA were amplified in parallel

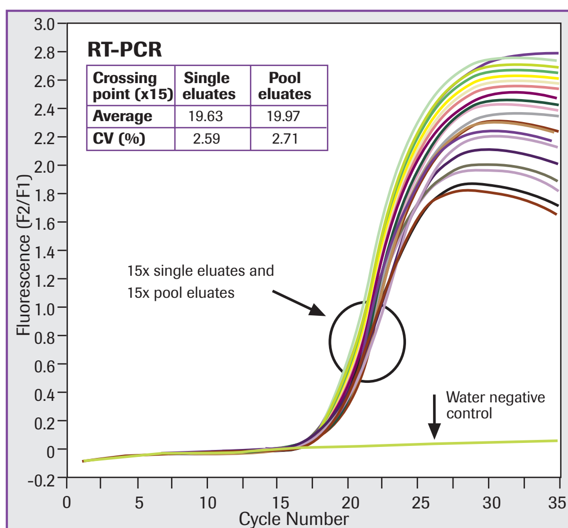


Figure 6: Reproducibility analysis of mRNA isolated from 10 mg mouse liver. mRNA was isolated from 15 x 10 mg mouse liver (single eluates) and analyzed by RT-PCR using the cyclophilin-A as target transcript. In order to discriminate between variation due to the LightCycler Instrument or due to the whole instrument system (MagNA Pure LC and LightCycler), LightCycler analysis was carried out using as template the 15 single eluates and a pool of all single eluates (pool eluate) in 15 replicates. Coefficient of variance (CV) of the crossing point (cps) were calculated

Test for DNA contamination

mRNA was isolated from different amounts of mouse tissue (spleen and liver) using the Ribolyser as homogenization method and used to amplify the cyclophilin-A gene in order to check for possible DNA contamination. A minus RT control PCR without reverse transcriptase was applied to the mRNA purified from spleen and liver (Figure 5). Both results of the minus RT control PCR confirmed the absence of DNA contamination in mRNA samples since the LightCycler graphs showed flat lines.

These results confirm the high quality of the mRNA isolated with the MagNA Pure LC mRNA Isolation Kit II (Tissue). Applying quantitative RT-PCR in precise gene expression analysis is crucially depending on DNA-free mRNA.

Intra-assay variance analysis

mRNA was isolated from 15 x 10 mg mouse liver (single eluates) and analyzed by RT-PCR using the cyclophilin-A transcript as target. In order to discriminate between variations due to the LightCycler Instrument or due to the whole instrument system (MagNA Pure LC and LightCycler), LightCycler analyses were carried out using the 15 single eluates and a pool of all single eluates (pool eluate) in 15 replicates as templates (Figure 6). Coefficient of variance (CV) of the crossing point (cps) were calculated. The CV % values were always below 3 %. Moreover, no significant difference was noticed between single and pooled eluate CV values: 2.59 % and 2.71 %, respectively.

Both results confirm that the MagNA Pure LC Instrument together with the MagNA Pure LC mRNA Isolation Kit II (Tissue) provides high reproducible data and does not cause any variation with the analytic system.

Conclusion

The performance of the MagNA Pure LC mRNA Isolation Kit II (Tissue) on the MagNA Pure LC Instrument is described in this article. mRNA was isolated from different amounts and types of frozen mouse tissue (1 - 10 mg spleen, liver, or kidney). The analysis of the isolated mRNA using different tests demonstrated that the applied purification process yields nucleic acids of a high quality with amounts reflecting the amounts of starting material. The isolated mRNA was free of DNA due to the efficient DNase digestion step. This performance in isolating mRNA from frozen tissue makes the MagNA Pure LC mRNA Isolation Kit II (Tissue) together with the MagNA Pure LC Instrument an accurate and reliable routine tool in the field of gene expression analysis.

References

1. Farrell, R. E. (1993), RNA Methologies: A Laboratory Guide for isolation and characterization, Academic Press, San Diego.

Product	Pack Size	Cat. No.
MagNA Pure LC	1 instrument plus accessories	2 236 931
MagNA Pure LC mRNA Isolation Kit II (Tissue)	1 kit (192 reactions)	3 172 627

http://biochem.roche.com/magnapure