Tissue Homogenization with the MagNA Lyser Instrument for Total RNA Extraction Using the TriPure Reagent

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Introduction

The MagNA Lyser Instrument is used for automated disruption of tissues or other biological materials prior to nucleic acid (NA) purification. It is optimized for use with the MagNA Pure LC Extraction Kits and integrates perfectly into the PCR Workflow System of Roche Applied Science: the MagNA Pure LC System for extraction of nucleic acids, and the LC Carousel Centrifuge and LightCycler System for real-time PCR.

The basic principle of cell disruption with the MagNA Lyser is the fast-moving, oscillating reciprocal motion of the rotor. Cell disruption is caused by the collision of ceramic beads with the sample.

Table 1: **RNA quantity and purity after addition of RNA Later Solution and TriPure extraction**

Liver (mg)	100	100	100	100
RNA Later (ml)	–	–	0.5	1
TriPure (ml)	0.5	1	0.5	0.5
total RNA (µg/µl)*	0.429	0.381	0.203	0.408
$A_{260\,nm}/A_{280\,nm}$	1.88	1.86	2.03	1.99
Spleen (mg)	80	80	–	80
RNA Later (ml)	–	–	–	1
TriPure (ml)	0.5	1	–	0.5
total RNA (µg/µl)*	0.198	0.328	–	0.205
$A_{260\,nm}/A_{280\,nm}$	1.87	1.89	–	1.83
Pancreas (mg)	80	80	80	–
RNA Later (ml)	–	–	0.5	–
TriPure (ml)	0.5	1	0.5	–
total RNA (µg/µl)*	0.6	0.429	0.519	–
$A_{260\,nm}/A_{280\,nm}$	1.61	1.88	1.94	–
Gut (mg)	40	40	–	40
RNA Later (ml)	–	–	–	1
TriPure (ml)	0.5	1	–	0.5
total RNA (µg/µl)*	0.31	0.587	–	0.43
$A_{260\,nm}/A_{280\,nm}$	1.83	1.66	–	1.91
Tail (mg)	20	20	20	–
RNA Later (ml)	–	–	0.5	–
TriPure (ml)	0.5	1	0.5	–
total RNA (µg/µl)*	0.059	0.405	0.542	–
$A_{260\,nm}/A_{280\,nm}$	2	1.86	1.7	–

*all samples were diluted in equal volumes of DEPC-treated water

The aim of this work was to assess the efficacy of the TriPure Reagent in combination with the MagNA Lyser Instrument, to extract total RNA from tissue samples. Tripure Isolation Reagent allows the isolation of total RNA, DNA, and protein from the same sample in single-step liquid-phase separation. This reagent performs well with both small and large quantities of tissue or cells of human, animal, plant, or bacterial origin. We used the classical TriPure solution – which contains phenol and guanidine thiocyanate – together with the MagNA Lyser Instrument to broaden the application of both devices.

Materials and Methods

Animals

Eight-week-old female C57Bl/6 mice were purchased from Charles River (Font Saint Landry, Brussels, Belgium). Animals were maintained in our animal facilities on standard laboratory chow.

Isolation of total RNA

Tissue specimens were either used directly, frozen in liquid nitrogen after collection, or stored in RNA Later Solution (Qiagen Benelux, Netherlands). The RNA Later Solution protects RNA in animal tissues and other biological samples, allowing transport and storage at room temperature. Total RNA was extracted using the TriPure Solution as described in the manufacturer's protocol. The processing conditions for the MagNA Lyser were the following: for liver, pancreas, gut and spleen, one run of 50 s at 6,500 rpm; for the tail, three runs of 30 s at 6,500 rpm.

Quantity and purity of total RNA

RNA was quantified measuring absorbance at 260 nm ($A_{260\,nm}$) and RNA purity was determined by the ratio $A_{260\,nm}/A_{280\,nm}$ using a classical spectrophotometer.

Quantification of mRNA by real-time RT-PCR of classical housekeeping β-actin gene

First, reverse transcription (RT) was performed with the Transcriptor Reverse Transcriptase according to the kit instructions. The classical housekeeping gene β-actin was used to quantify the amount of mRNA (forward and

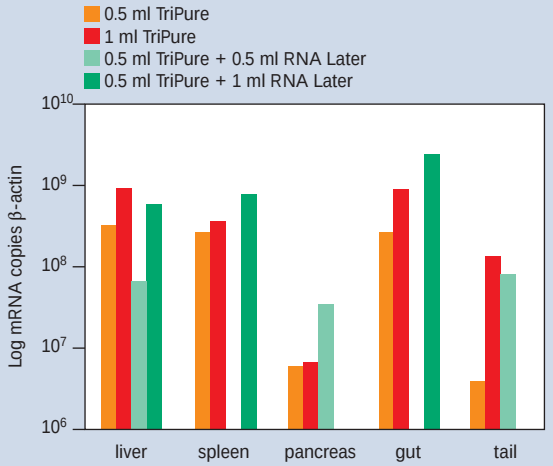

Figure 1: Number of β-actin mRNA copies in different murine tissues. Samples were stored in the presence or absence of RNA Later and extracted in different volumes of TriPure Reagent.

reverse primers and probe sequences are respectively 5′-TCCTGAGCGCAAGTACTCTGT-3′, 5′-CTGATCCACATC-TGCTGGAAG-3′, and 5′-ATCGGTGGCTCCATCCTGGC-3′). The LightCycler FastStart DNA Master Hybridization Probes Kit was used according to the instructions: 300 nM probes, 300 nM of each primer, and 25 mM $MgCl_2$ were applied. Amplification was performed under the following cycle conditions: a pre-incubation step for 10 min at 95°C, followed by 45 cycles of 0 s at 95°C, 20 s at 60°C (temperature ramp was 20°C/s).

Results and Discussion

To measure the amount of total RNA and mRNA extracted using the MagNA Lyser Instrument and the TriPure Reagent, we measured the absorbance at 260 nm and performed quantitative real-time RT-PCR of β-actin using the LightCycler Instrument. Interestingly, we observed that using the TriPure Solution with the MagNA Lyser Instrument allows extraction of large quantities of RNA and mRNA from different mouse tissues such as liver, spleen, pancreas, gut, and tail (Table 1, Figure 1). The ratio of $A_{260\,nm}/A_{280\,nm}$ shows that high-quality DNA/RNA was extracted from these tissues at the same time.

We demonstrated that adaptation of the TriPure Reagent volume – as described in the package insert – enhanced the efficacy of the extraction. The use of RNA Later to stabilize the RNA during the extraction procedure seemed to be efficient for tissue containing a large amount of RNase such as pancreas. In the other tissue samples, the TriPure Reagent alone was sufficient for stabilization. ■

Order INFO

Product	Pack Size	Cat. No.
TriPure Isolation Reagent	50 ml	11 667 157 001
	200 ml	11 667 165 001
Transcriptor Reverse Transcriptase	250 U (25 reactions)	03 531 317 001
	500 U (50 reactions)	03 531 295 001
	2,000 U (200 reactions)	03 531 287 001
LightCycler FastStart DNA Master Hybridization Probes	1 kit (96 reactions)	03 003 248 001
	1 kit (480 reactions)	12 239 272 001
MagNA Lyser Instrument	1 instrument (110 V)	03 358 968 001
	1 instrument (220 V)	03 358 976 001
MagNA Lyser Green Beads		03 358 941 001