RNAzol® RT: a new single-step method for isolation of RNA

The single-step RNA isolation with RNAzol® RT yields RNA ready for RT-PCR without additional purification or DNase treatment. Previous single-step methods required additional steps to remove DNA contamination before the isolated RNA was used in RT-PCR. RNAzol RT simplifies the single-step method and adds to its versatility and effectiveness. RNAzol RT isolates pure and undegraded RNA with protocols yielding either a single fraction containing total RNA or two separate fractions containing mRNA and small RNA.

RNAzol RT (US patent[1]) is an advanced version of the single-step RNA isolation reagent[2] that provides improved purity and yield of isolated RNA. Its predecessor, RNAzol, was introduced in 1989 as the first reagent employing the single-step method of RNA isolation based on acid guanidinium thiocyanate–phenol–chloroform extraction[3]. Over the last two decades, we introduced a series of reagents based on this method, including TRI Reagent®[4].

In the new approach to the single-step method, the RNAzol RT protocol does not employ a chloroform-induced phase separation to obtain pure RNA. RNAzol RT separates RNA from other molecules in a single step based on the interaction of phenol and guanidine with cellular components. A biological sample is homogenized or lysed in RNAzol RT, and the homogenate is supplemented with water. This addition of water induces the precipitation of DNA, proteins and polysaccharides while RNA remains soluble in the homogenate. The precipitated compounds are removed from the homogenate by centrifugation. Pure RNA is precipitated from the resulting supernatant by the addition of alcohol, followed by RNA washing and solubilization. The whole procedure is performed at room temperature, including centrifugation, and can be completed in less than 1 h.

RNAzol RT isolates pure and undegraded RNA that is ready for use without DNase treatment. The isolated RNA can be used for RT-PCR, qRT-PCR, microarrays, poly(A)$^+$ selection, northern blotting, RNase protection assays and other molecular biology applications.

There are two protocols for isolation of RNA using RNAzol RT. The first protocol isolates total RNA containing all classes of RNA in a single fraction. The second protocol size-separates cellular RNA into (i) an mRNA fraction with RNA longer than 200 bases, containing ribosomal RNA and mRNA, and (ii) a small-RNA fraction with RNA shorter than 200 bases, containing small ribosomal RNA, tRNA and microRNA. An optional extraction step using 4-bromoanisole (BAN) can be employed for samples containing excessive amounts of polysaccharides or other undesired contaminants. It is also possible to isolate DNA from the RNAzol RT extract (see RNAzol RT protocols at www.mrcgene.com).

Typically, the extraction of animal-derived samples with RNAzol RT yields RNA with an $A_{260/280}$ ratio of 1.9–2.0 and a RIN value of 8–9.8. Processing of RNA from more challenging plant-derived samples yields RNA with an $A_{260/280}$ ratio of 1.8–1.9 and a RIN value of 7–8.

Figure 1 depicts the integrity of RNA isolated from rat liver using the two RNAzol RT protocols outlined above. The isolated RNA was analyzed by electrophoretic separation using the Agilent RNA 6000 Nano™ and small-RNA kits. The total RNA fraction contains ribosomal RNA, mRNA and small RNA and has a RIN value of 9.3. In this total RNA, the small RNA comprises about 15%, a typical amount for animal-derived tissues. The mRNA fraction contains ribosomal RNA and mRNA and has a RIN value of 9.6. This fraction is virtually depleted of small RNAs. The small-RNA fraction consists mostly of small ribosomal RNA and tRNA. The microRNA content in this fraction is visualized in the electrophoretic profile with an expanded *y*-axis.

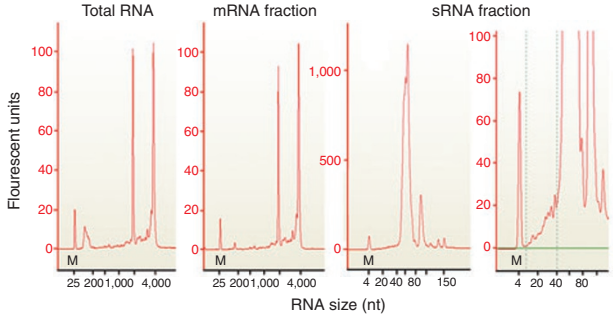

Figure 1 | Electropherograms of RNA isolated with RNAzol RT using protocols for: total RNA, mRNA fraction and small-RNA fraction. The last panel shows the small-RNA fraction with expanded ***y***-axis. Electrophoresis was performed in the Agilent 2100 Bioanalyzer™ using the RNA 6000 Nano Kit or the Small RNA Kit with 140 ng of total RNA or mRNA fraction and 14 ng of small-RNA fraction. M, marker.

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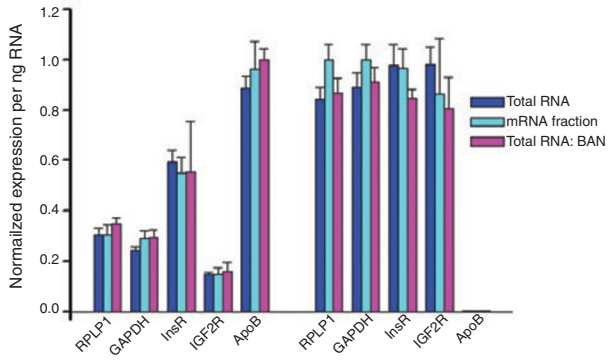

Figure 2 | Comparison of normalized mRNA expression in RNA isolated with RNAzol RT from rat liver and kidney using protocols for: total RNA, mRNA fraction and total RNA with BAN extraction. Quantitative RT-PCR was performed with primers for RPLP1, GAPDH, insulin receptor, IGF2 receptor and apolipoprotein B mRNA.

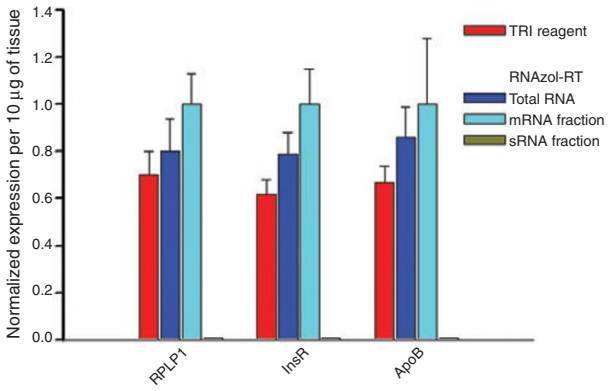

Figure 3 | Comparison of the normalized mRNA expression in RNA isolated by RNAzol RT and TRI Reagent from 10 µg of rat liver. A standard phase-separation protocol was used with TRI Reagent. With RNAzol RT, the protocols for total RNA isolation and isolation of mRNA fraction and small-RNA fraction were used. The amounts of RPLP1, insulin receptor and apolipoprotein B mRNA were evaluated by qRT-PCR.

This profile shows the presence of microRNA ranging in size from 8 to 40 nucleotides.

Figure 2 shows the mRNA content in RNA isolated from rat liver and rat kidney using three different protocols: isolation of total RNA, isolation of an mRNA fraction, and isolation of total RNA with BAN extraction. The mRNA content is calculated as a normalized mRNA expression per ng of RNA. Quantitative RT-PCR was performed for mRNAs ranging in size from 0.5 kb to 14 kb: RPLP1 (522 b), GAPDH (1,307 b), insulin receptor (5,397 b), IGF2 receptor (8,810 b) and apolipoprotein B (14,091 b). This comparison shows that all three protocols provide RNA with an equal content of mRNA, as calculated per ng of total RNA.

Control experiments showed that the RNA isolated by RNAzol RT can be used in RT-PCR without additional purification or treatment with DNase. PCR performed with RNA samples without reverse transcription showed no amplification of DNA in a 40-cycle PCR. Also, **Figure 2** shows no amplification product when kidney-derived cDNA was used in PCR with liver-specific apolipoprotein B primers (encompassing a single exon sequence, 5,565 b–5,686 b). Thus, the RNAzol RT procedure effectively removes DNA from the isolated RNA.

A comparison of the effectiveness of RNAzol RT and TRI Reagent to isolate RNA is shown in **Figure 3**. The amounts of RPLP1, insulin receptor and apolipoprotein B mRNAs in rat liver were evaluated by qRT-PCR. In order to evaluate RNA recovery with these two reagents, the mRNA amount is presented as a normalized mRNA expression per 10 µg of tissue. The amount of mRNA isolated from the same tissue weight is consistently higher for RNAzol RT as compared to TRI Reagent. This reflects a 10–15% higher recovery of total RNA when tissues are processed with RNAzol RT.

Additionally, **Figure 3** shows no detectable mRNA expression in the small-RNA fraction (fourth bar for each amplified mRNA), thereby demonstrating the absence of mRNA in the small-RNA fraction. Thus, the RNAzol RT procedure effectively separates small RNA from the larger RNA molecules.

For more than two decades, single-step RNA isolation has been employed as a very dependable method for RNA isolation. In order to use the isolated RNA for RT-PCR, the previous single-step methods required additional steps to remove DNA contamination. With the introduction of RNAzol RT, the single-step method has been improved to provide RNA that is ready to use for RT-PCR without additional purification or treatment with DNase. RNAzol RT further simplifies the single-step method, adding to its versatility and effectiveness. RNAzol RT isolates pure and undegraded RNA with a protocol for yielding a single fraction containing total RNA, or with a protocol yielding enriched mRNA and small-RNA fractions. These RNAzol RT protocols can be applied successfully to the most demanding applications, including the isolation of RNA from specimens containing large quantities of extracellular material, such as those from plants.

1. Chomczynski, P. Reagents and methods for isolation of purified RNA. US Patent 7,794,932 (2010).
2. Chomczynski, P. & Sacchi, N. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156–159 (1987).
3. Chomczynski, P. Product and process for isolating RNA. US Patent 4,843,155 (1989).
4. Chomczynski, P. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. *Biotechniques* **15**, 532–534 (1993).