RNAzol® BD: a reagent for the effective isolation of RNA from whole blood

Previously, we introduced an advanced version of the single-step method for RNA extraction. We have now adapted this methodology for the isolation of total RNA, mRNA, small RNA and microRNA from whole blood, plasma or serum of human and animal origin. This new reagent, RNAzol® BD, provides the highest yield and purity of blood-derived RNA, and it also allows for the simultaneous isolation of DNA from samples used for RNA isolation.

Introduction

RNAzol® BD is a monophasic solution containing acidic phenol and guanidine thiocyanate. This reagent is an improvement over previous reagents based on the single-step method of RNA isolation[1–5]. A blood, plasma or serum sample is lysed, and the RNA is stabilized in RNAzol® BD. The lysates can be processed immediately or stored at room temperature for 1 day, 2–3 days at 4 °C and for months or years at –20 °C or –70 °C. Researchers can use 1 ml of RNAzol® BD to process 0.5 ml of blood in less than 90 minutes. The isolated RNA is pure, undegraded and can be used directly, without DNase treatment, for RT-PCR and other molecular biology applications. The RNAzol® BD protocol also allows for the isolation of RNA and DNA from the same blood sample.

There are two protocols for the isolation of RNA using RNAzol® BD. The first protocol isolates total RNA with all classes of RNA in a single fraction. The second protocol separates RNA into (i) an 'mRNA fraction' containing ribosomal RNA and mRNA, and (ii) a 'small-RNA fraction' (<200 bases), containing small ribosomal RNA, transfer RNA and microRNA (miRNA).

RNAzol BD performance

Using RNAzol® BD, processing human blood with a normal count of white cells typically yields 8–22 µg of total RNA/ml of blood, with an $A_{260/280}$ ratio of 1.8–2.0 and RIN values of 6.5–8.3. Isolation of the mRNA fraction yields 5–13 µg RNA/ml blood with RIN values of 7.0–8.5. Isolation of the small-RNA fraction yields 2–5 µg RNA/ml of blood. Efficiency of RNAzol® BD in recovery of total RNA was tested by supplementation of human blood lysate with exogenous RNA. The recovery of the added RNA ranged from 97% to 102%. The DNA yield from samples used for RNA isolation is 40–60 µg of DNA/ml of blood. Assuming a cellular DNA content of 6.66 pg/cell, this yield would correspond to 6–9 million nucleated cells per ml of blood. Examples of RNA and DNA recovery from human blood are shown in **Table 1**.

The integrity of the total RNA, mRNA and small-RNA fractions isolated with RNAzol® BD from human blood is illustrated in **Figure 1**. In the electrophoretic profile of total RNA, the small RNA comprises about 40%. In our tests, the amount of small RNA recovered from human blood ranged from 18% to 42% of the total RNA. The mRNA fraction contains ribosomal RNA and mRNA, and it is virtually depleted of small RNA (<200 bases). The small-RNA fraction consists mostly of small ribosomal RNA and transfer RNA. The presence of miRNA in this fraction can be demonstrated by RT-qPCR (**Fig. 2**).

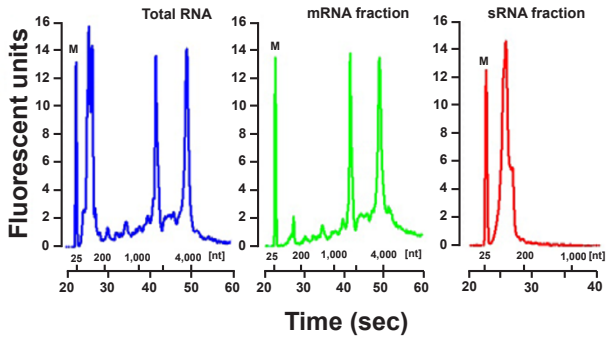

Figure 1 | Electropherogram of RNA isolated with RNAzol® BD from human blood. Agilent Bioanalyzer electropherograms of RNA isolated from human whole blood with RNAzol® BD using the protocols for total RNA, mRNA and the small-RNA (sRNA) fractions. The RNA was analyzed with the RNA 6000 Nano Kit. M, marker; nt, nucleotide.

The effectiveness of RNAzol® BD to isolate a range of RNA transcripts from whole blood is shown in **Figure 2**. Recovery of miR24 (22 bp), S100A12 (466 bp), GAPDH (1,425 bp), EPB42 (2,554 bp) and ITGA4 (6,082 bp) was evaluated in the total RNA, mRNA and small-RNA fractions. A cDNA equivalent of 1.25 ng of RNA was

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APPLICATION NOTES

Table 1 | Recovery of RNA and DNA from human blood using RNAzol BD.[a]

Subject	RNA		DNA	
	µg/ml	$A_{260/280}$	µg/ml	$A_{260/280}$
Male	21.3	1.96	44.9	1.83
Male	10.2	1.92	60.0	1.86
Male	12.7	1.97	40.2	1.81
Female	14.3	1.99	61.0	1.88
Female	10.4	1.98	41.3	1.81
Female	20.9	1.98	55.8	1.83

[a]Total RNA was isolated from 1 ml aliquots of frozen (–70 °C) human blood using 2 ml of RNAzol® BD. Following the recovery of RNA from the aqueous phase, the remaining phenol phase/interphase was used to isolate genomic DNA.

used for qPCR. No DNA contamination was detected in control PCR. As expected, the mRNA fraction contains 60–70% higher mRNA amounts than the total RNA fraction. A minuscule amount of degraded messenger RNA was occasionally detected in the small-RNA fraction. No miR24 was detected in the mRNA fraction. In addition, **Figure 2** compares the recovery of mRNA in total RNA isolated from blood using RNAzol® BD and TRI Reagent® BD. RNAzol® BD is 20–30% more effective than TRI Reagent® BD.

Our results using RNAzol® BD for the isolation of total RNA from 22 human whole-blood samples show that average recovery was 13.9 µg RNA/ml and the range was 8–22 µg RNA/ml. To account for the vastly different levels of RNA in the circulation, differences in the accumulation of gene transcripts should be normalized to a fixed volume of whole blood and not to a fixed quantity of RNA. Alternatively, RNA content could also be expressed per genomic DNA content in a blood sample.

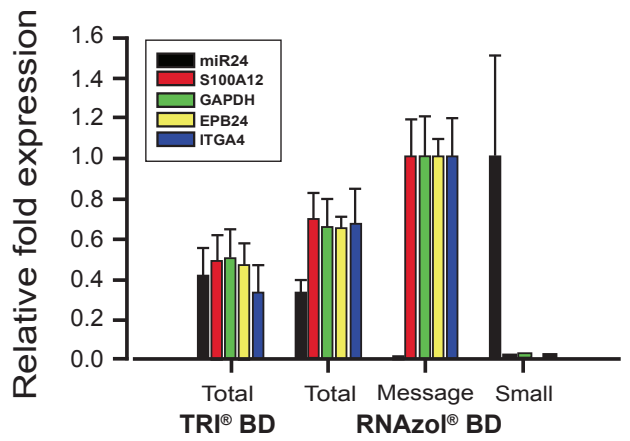

Figure 2 | Effectiveness of RNA isolation with RNAzol® BD. Whole blood was extracted from four individuals using RNAzol® BD or TRI Reagent® BD protocols for total RNA, mRNA and small-RNA fractions. A total of 50 ng of RNA was reverse transcribed, and miR24, S100A12, GAPDH, EPB24 and ITGA4 gene expression was evaluated by qPCR using cDNA equivalent to 1.25 ng RNA per assay.

Conclusion

To date, we have been unable to find reports that specifically cite the RNA levels in human whole blood. Occasionally, authors cite methods that claim to isolate 2–5 µg RNA/ml of human blood[6]. Evaluation of accumulation of a gene transcript may be subject to significant error when an RNA isolation method yields only a small percentage of the RNA content in a blood sample.

It remains to be determined to what extent the RNA content in blood is affected by the genetic makeup, disease state, physiological status and age of the donor. RNAzol® BD provides a new tool to study RNA transcripts in blood by effectively isolating a whole complement of RNA transcripts including mRNA, small RNA and miRNA.

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