Application of RiboPure™ Blood Kit on RNA purification from blood samples stabilized in RNAgard® Blood Tubes

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Introduction

RNAgard® Blood Tube (Cat No 62201-131 & 62201-141, Biomatrica) is a whole blood collection device that stabilizes the RNA present in blood cells and allows for ambient temperature handling and storage of the blood specimen. Purification of RNA from blood stabilized in RNAgard Blood Tubes was optimized with the BioMaxi® Blood RNA Purification Kit (Cat No 64201-601, Biomatrica). In order to expand application of the RNAgard Blood Tubes, we evaluate another blood RNA isolation kit to provide customers with an alternative for small volume RNA isolations. In this study, RiboPure™ Blood Kit (Cat No AM1928, Life Technologies) is optimized for 500µl to 1350µL of blood/stabilizer in the RNAgard Blood Tubes. We evaluate the quality of RNA obtained from using RiboPure Blood kit by Agilent 2100 Bioanalyzer. In addition, we assess changes in the relative expression of a panel of 34000 genes in whole blood specimens stabilized in RNAgard Blood Tubes over the course of 7 days. Our results demonstrate that high quality RNA from blood stabilized in RNAgard Blood tubes can also be purified by RiboPure Blood Kit.

Materials and Method

Blood sample processing

Human whole blood was collected in RNAgard Blood Tubes and stored at ambient room temperature (RGB). Control samples (-80˚C) and samples that were not protected (NP) by stabilizer were collected in K_2-EDTA-treated VACUETTE® tubes. Control samples were stored at -80°C and the NP samples were stored at ambient room temperature. RNA was isolated using RiboPure™ Blood Kit. All blood samples were analyzed at day 3 and/or day 7.

RNA purification

1. Invert RNAgard Blood Tube samples 3-5 times prior to commencing the extraction procedure to obtain a homogenous re-suspension of any precipitates in the blood stabilizer mixture.
2. Transfer up to 1.4mL blood/stabilizer mixture from RNAgard Blood Tube to a 2mL microfuge tube.
3. Add 1/3 of the sample volume of RNase-free water and vortex vigorously for 15 seconds.
4. Centrifuge 5 minutes at 12000-14000 rpm and carefully decant the supernatant without disturbing the pellet.
5. Add 800µL of RiboPure™ Blood Lysis Solution and 50µl of RiboPure Blood Sodium Acetate Solution to the pellet, and vortex vigorously for 15 seconds.
6. Transfer the sample to a 2mL microfuge tube and proceed to the extraction with Acid-Phenol:Chloroform, following the RiboPure Blood Kit protocol from “Section B, Cell Lysis and Initial RNA Purification, Step 3”. Follow according to the manufacturer’s instructions.

Note: If the pellet is larger than 1mL in Step 5 above, divide the sample into two 2mL microfuge tubes, adjust the volume to 1mL with RiboPure Blood Lysis Buffer, vortex briefly and proceed with Acid-Phenol:Chloroform extractions. Samples can then be combined and processed through the same purification column after addition of the adjusted volume of 100% ethanol.

RNA analysis

Total RNA, extracted from blood samples as above, was quantified by absorbance spectroscopy. RNA integrity and quality was assessed by Agilent 2100 Bioanalyzer and RNA 6000 Nano Kit. Gene expression profile was assessed using the Illumina Human HT-12 gene expression array according to the manufacturer's instructions.

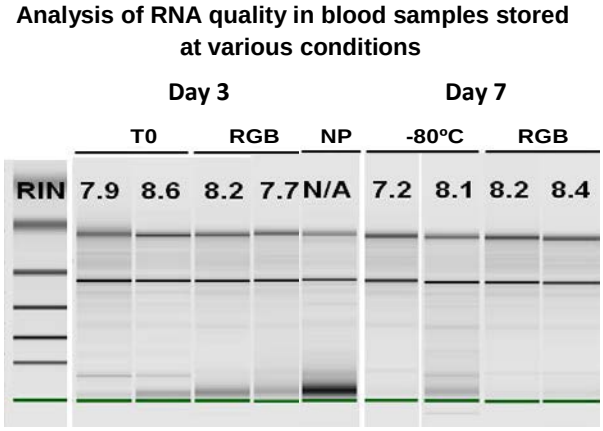

Figure 1. Analysis of RNA quality in blood samples stored at various conditions using the Agilent 2100 Bioanalyzer. Gel image shows the integrity of RNA extracted by RiboPure™ Blood Kit at day 3 and day 7. The samples include fresh samples (T0), samples collected in RNAgard Blood Tubes (RGB), samples stored at -80°C (-80°C), and non-protected samples at room temperature (NP). Numbers above each lane represent RNA Integrity Number (RIN).

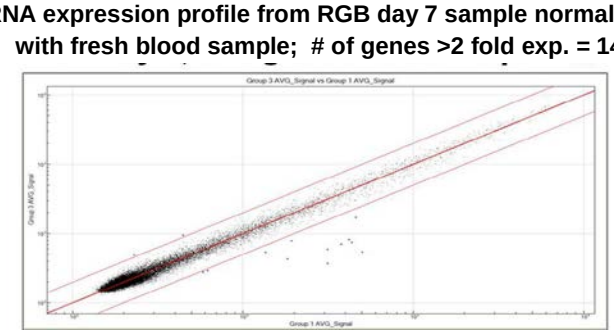

Figure 2. Analysis of gene expression profile of 34000 genes using Illumina Human HT12 Bead Array. RNA is purified by RiboPure Blood Kit. Expression profiles of RNA from blood stored in RNAgard Blood Tubes for 7 days at room temperature are identical to that of RNA from fresh blood.

Results and Discussion

In this study, we evaluate application of RiboPure Blood Kit on RNA purification from blood samples stabilized in RNAgard Blood Tubes. Our results demonstrate that RiboPure Blood Kit is compatible with RNAgard Blood Tubes. High quality RNA can be purified by RiboPure Blood Kit from human blood samples stabilized in RNAgard Blood Tubes (Figure 1). By comparison with fresh blood samples, gene expression profile remains unaltered in blood samples stored in RNAgard blood tubes at room temperature for 7 days (Figure 2).

Conclusion

In addition to BioMaxi Blood RNA Purification Kit, RiboPure Blood Kit is an alternative RNA purification approach for blood samples stored in RNAgard blood tubes at room temperature.

***Note**: Please read all instructions for the RNAgard Blood System prior to using this protocol.*

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