

RNAgard® Blood System (RUO) Application Note

Introduction

Compared to many commonly assayed cellular biomolecules, RNA is particularly susceptible to degradation, primarily due to the ubiquitous presence of RNases. The instability of RNA often results in poor accuracy and reproducibility of RNA assays. Moreover, it typically requires storage and shipment at temperatures $<-20^{\circ}\text{C}$, which is expensive due to freezer energy costs and dry ice shipment costs. To address these issues, the RNAgard Blood System was designed to inhibit degradation of cellular RNA during collection and transport of RNA in whole blood for 14 days at room temperature and 30 days at 4°C . The accompanying BioMaxi™ RNA Purification Kit was optimized to provide maximum quality and yield upon RNA isolation. The following studies were performed to evaluate the effectiveness of the RNAgard Blood System in stabilizing RNA in whole blood and purifying the RNA for subsequent analysis.

RNAgard Blood System is for Research Use Only. Not for use in diagnostic procedures.

14-day RNA protection at ambient temperatures

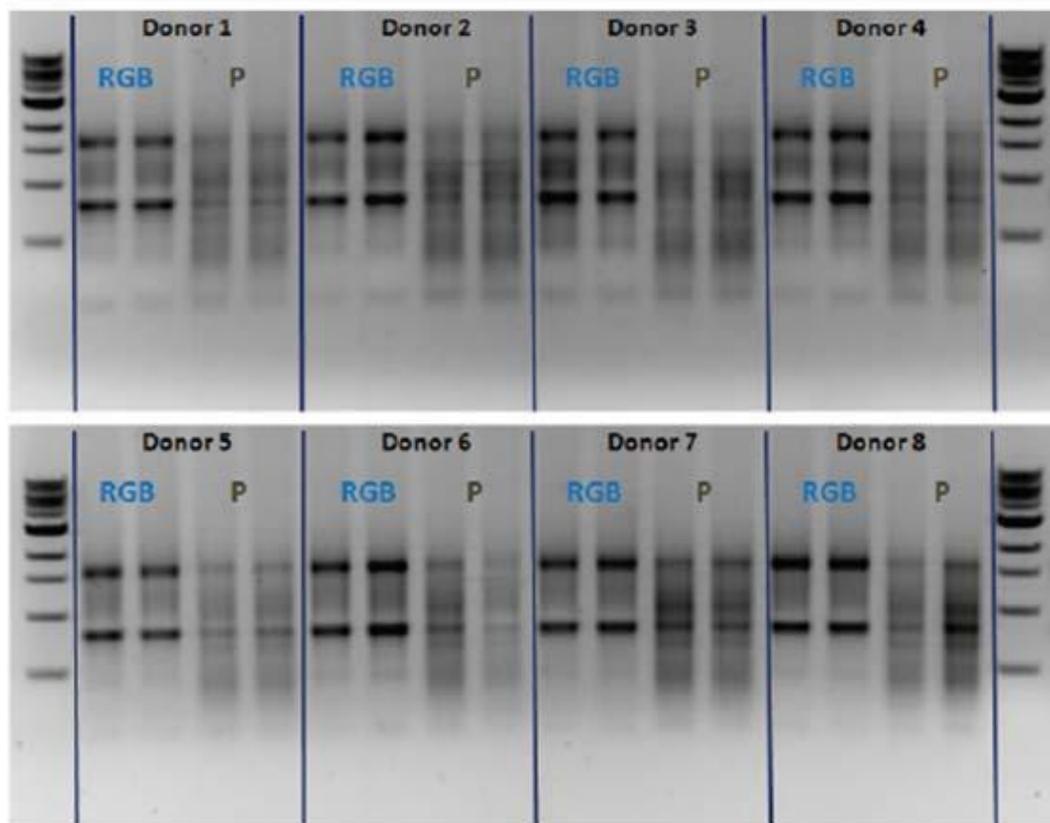


Figure-1: Experiment was designed to collect blood from 8 different donors. Blood was collected in RNAgard Blood Tubes and competitor blood stabilizing RNA tubes. Tubes were left out at ambient room temperature at 14 days. The BioMaxi blood RNA purification kit was used to isolate the RNA from the RNAgardblood tubes. Competitor P used competitor's blood RNA purification kit to isolate the RNA from competitor P blood tubes. 200ng of RNA was run on a 1.2% agarose gel.

RNA yield from BioMaxi Blood RNA Purification Kit

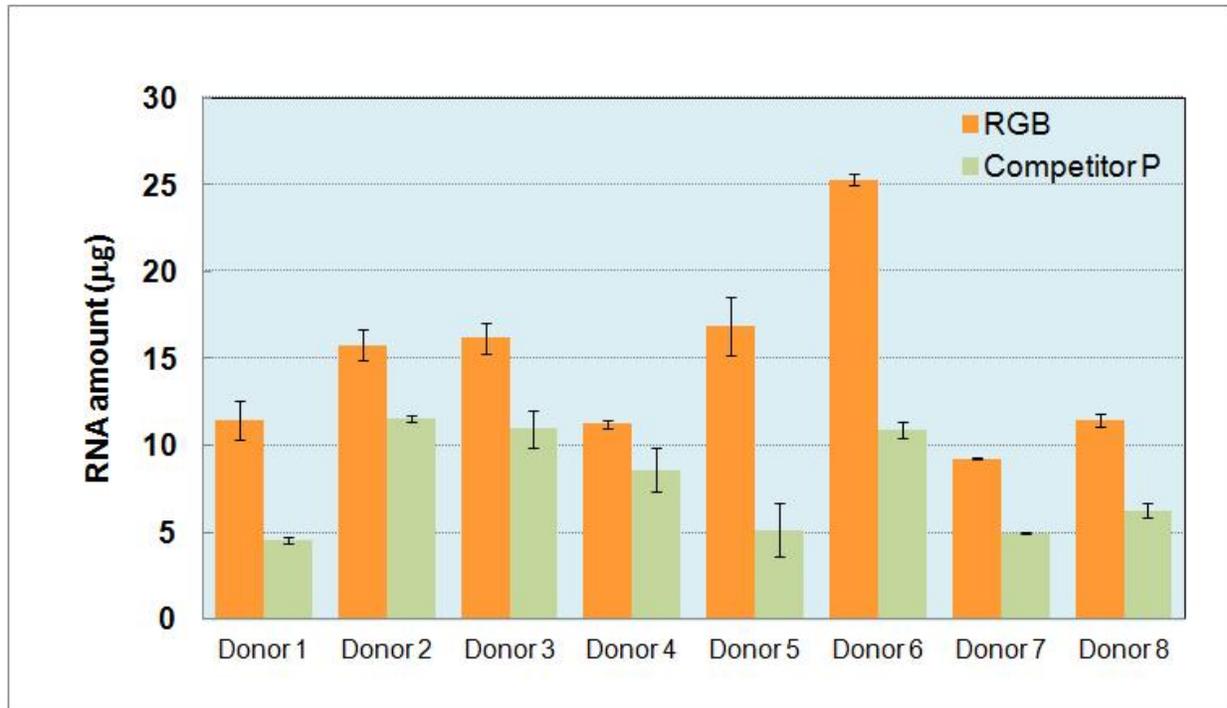


Figure-2: RNA yield analyzed by UV spectroscopy. RNA yield for the RNAgard Blood System (RGB) was compared to that from Competitor P using blood isolated from 8 different donors. RNA amounts are for whole tube (2.5 ml blood) isolation using the BioMaxi Blood RNA Purification kit after 14 days of storage at ambient temperature. The results show that RNA yield obtained using RGB was consistently higher than that obtained using Competitor P's RNA system at this time point.

RNA Stability after 7 weeks at 4°C

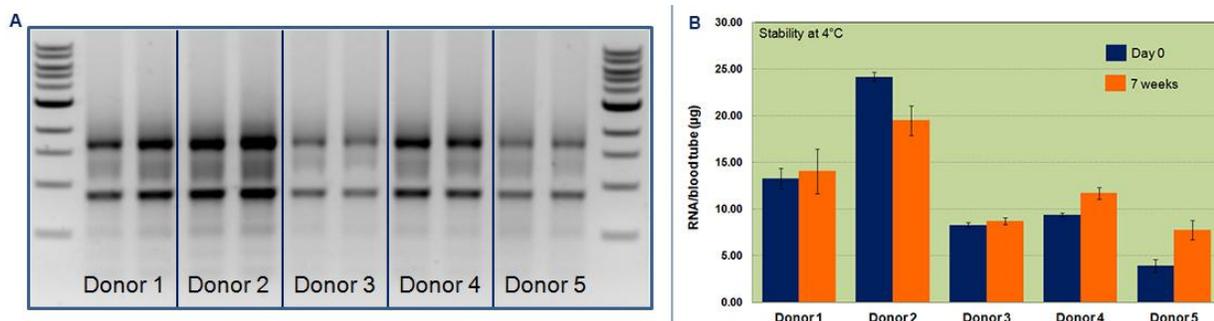


Figure-3: RNA yield of whole blood from 5 different donors was tested using the RNAgard Blood System following storage for 7 weeks at 4°C versus control (Day = 0). Data shows that the quality and yield of RNA following 7 weeks of storage in RNAgard Blood Tubes at 4°C was equivalent to that of RNA purified from whole blood on the day of blood draw.

Preservation of native RNA expression at ambient temperatures

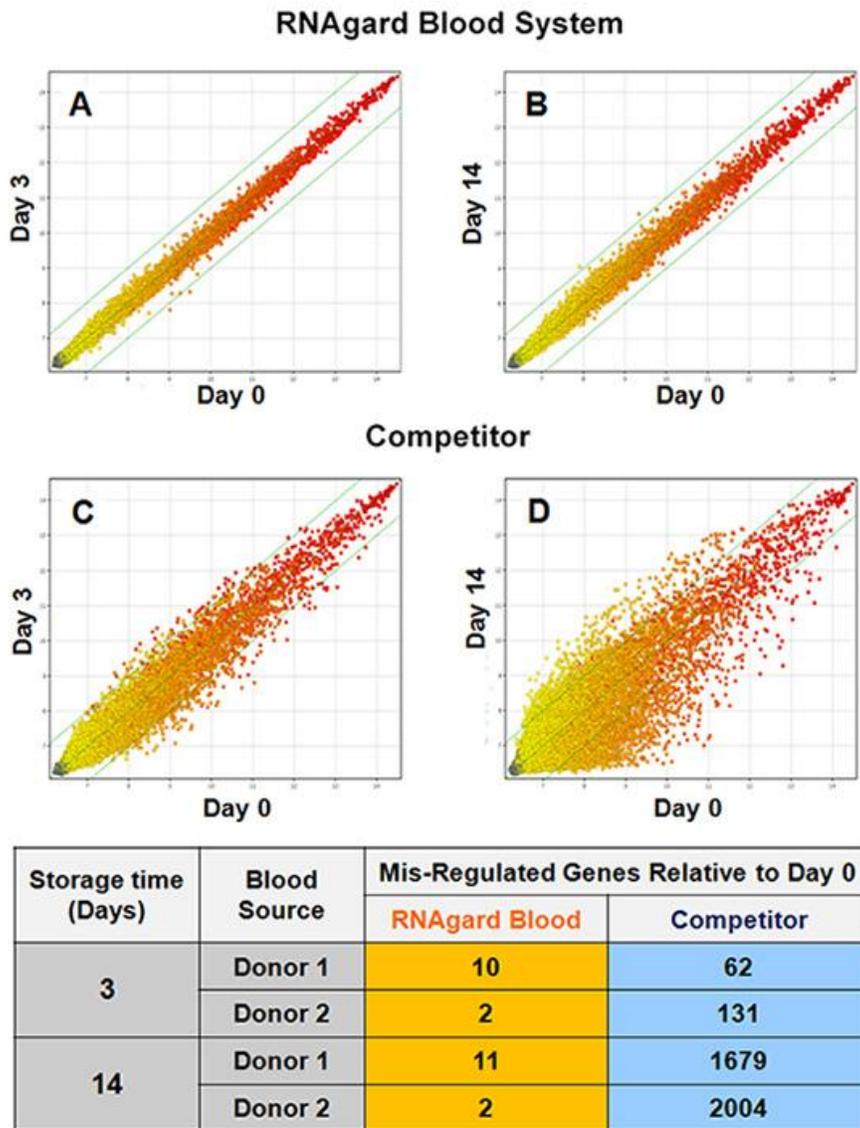


Figure-4: Gene expression analysis of blood RNA preservation after 3 and 14 days in RNAgard Blood (RGB). Human blood from 2 healthy donors was collected in RNAgard tubes or competitor's RNA preservation tubes. RNA was isolated on collection day and after 3 or 14 days of room temperature storage. Left: Representative analysis, for Donor 2, of gene expression profile of more than 47000 transcripts, using the Illumina Human HT12 Bead Array, for RNA extracted from blood collected in RNAgard Blood or competitor's Tubes after 3 days (A,C) or 14 days (B,D) of room temperature storage, relative to RNA extracted from freshly collected blood, using RNA isolation methods recommended for each product. Right: Table summarizing the microarray results. Displayed are the number of genes with > 2-fold change in expression levels relative to expression levels on the day of blood sample collection.

RNAgard Blood System: Reproducible and Repeatable RNA Purification: RNA Purity (A_{260}/A_{280}) for 3 Operators (A, B, C)

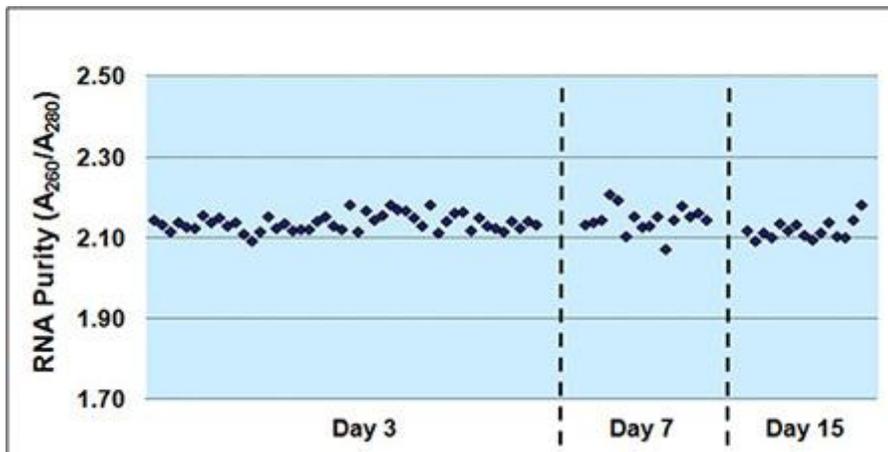


Figure-5: RNA purity obtained using the RNAgard Blood System provides is highly reproducible. Blood was collected from 8 healthy donors. After 3, 7 or 15 days of sample storage, RNA was purified from duplicate samples. RNA purity of each individual sample was determined by UV spectrophotometry (A_{260}/A_{280}). On Day 3, duplicate samples for each donor were purified by 3 different operators showing reproducibility of our system.

Stability of specific RNA transcripts isolated with RNAgard Blood System

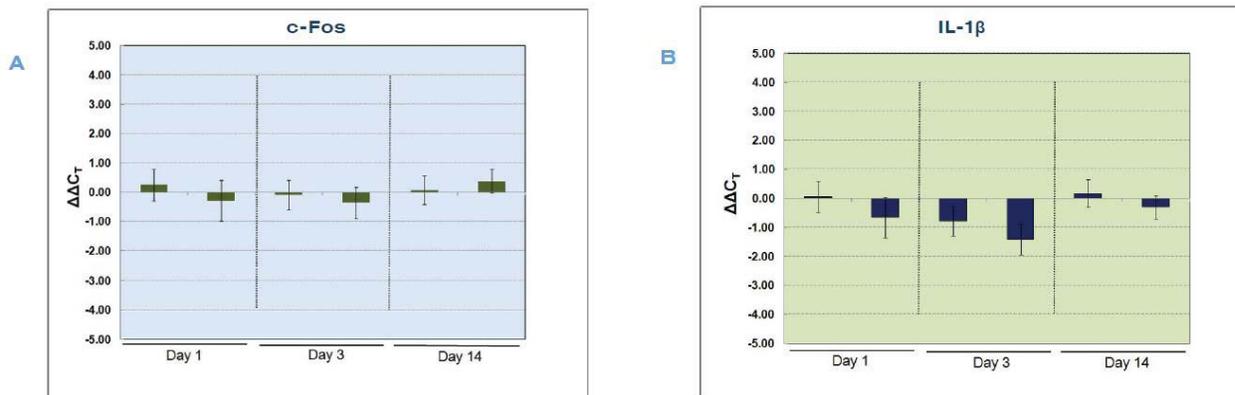


Figure-6: RNAgard Blood Tube maintains unaltered gene expression for 14 days at 18-25°C. Blood from two healthy donors was collected in RNAgard tubes and purified with BioMaxi RNA after the indicated storage time. Gene expression of C-Fos (A) and IL1- β (B), normalized to 18S rRNA, was determined by qPCR at the indicated storage time, relative to day 0 samples.

Discussion

The RNAgard Blood System is designed for the collection, preservation and purification of RNA from whole blood samples. The preservative reagent is designed to rapidly permeate and lyse cellular structures and membranes, and to stabilize and protect RNA from degradation. Preservation is effective for up to 14 days at room temperature and 1 month at 4°C. Purification with the BioMaxi RNA Purification Kit provides high quality and yield of RNA for downstream analysis. It maintains unaltered gene expression. Use of the RNAgard Blood System should significantly improve the accuracy of assay results compared to existing competitor products as well as reduce costs for sample transport.