RNA Purification from Biomatrica RNAgard® Blood Tubes Using the Maxwell® 16 LEV simplyRNA Blood Kit

ABSTRACT

Here we describe a research application for RNA extraction from blood samples using the Maxwell® 16 Instrument and the Maxwell® 16 LEV simplyRNA Blood Kit*. Samples were collected in Biomatrica RNAgard® Blood Tubes, and the Maxwell® 16 Instrument was configured with the Maxwell® 16 High-Strength Magnetic Rod and Plunger Bar Adaptor.*

**For Research Use Only. Not for use in diagnostic procedures.*

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INTRODUCTION

Labs studying RNA expression often face challenges when dealing with blood samples. Method of blood collection, storage and shipping conditions, and time between collection and analysis can all contribute to RNA degradation by RNases and unintentional expression of individual genes after blood collection. RNAgard® Blood Tubes are designed for collection and stabilization of RNA for gene expression analysis. The tubes immediately lyse whole blood cells and stabilize RNA, preserving global gene expression profiles (1).

The Maxwell® 16 LEV simplyRNA Blood Kit (a) is used with the Maxwell® 16 Instrument(a) configured with the LEV High-Strength Magnetic Rod and Plunger Bar Adaptor to purify RNA from whole blood. This RNA purification procedure is a simple method with minimal lysate handling before automated purification. The instrument processes up to 16 samples in about 1 hour, and the low elution volume generates concentrated, high-quality RNA suitable for use in common downstream applications. Here, we describe a research application of the simplyRNA Blood kit and the Maxwell® 16 Instrument for purifying RNA from blood collected in RNAgard® Blood Tubes.

MATERIALS AND METHODS

- RNAgard® Blood Tubes(a), Biomatrica Cat. #62201-131
- BioMaxi™ Precipitation Buffer(a), Biomatrica Cat. #RP1-001-FG
- Maxwell® 16 simplyRNA Blood Kit, Cat.# AS1310
- 50ml conical tubes
- Centrifuge

PROTOCOL

Blood samples were collected from four individuals and processed in triplicate using the following protocol: 2.5ml of blood was drawn into RNAgard® Blood Tubes. Samples A, B and C were drawn 4 hours prior to extraction. Sample D was drawn one day prior to RNA extraction. All tubes were shaken/inverted at least 5 times after blood was drawn.

Preprocessing of all tubes occurred as follows:

1. Sample was transferred to a 50ml conical tube.
2. 3ml of BioMaxi™ Precipitation Buffer was added.
3. The mixture was incubated at room temperature for 15 minutes, shaking at 550rpm.
4. The tube was then vortexed for 30 seconds so that the liquid touched the top of the tube.
5. The tube was centrifuged at room temperature for 30 minutes at 4500 *x g*.
6. Supernatant was decanted and pellet dried for 2 minutes.

Maxwell® 16 LEV simplyRNA Blood Kit Protocol

1. Add 200µl of simplyRNA Homogenization Solution + 2% 1-thioglycerol to each pellet. Vortex.
2. Add 200µl of simplyRNA Lysis Buffer to each sample. Vortex 20 seconds.
3. Add entire sample to Well #1 of the simplyRNA Blood Cartridge.
4. Add a plunger to Well #8 of the simplyRNA Blood Cartridge.
5. Add 10µl of DNase to Well #4 of the simplyRNA Blood Cartridge
6. Add 50µl of Nuclease-Free Water to each elution tube.
7. Process on the LEV→RNA→simplyRNA Blood protocol of the Maxwell® 16 Instrument.
8. Store RNA at - 80°C until ready to use.

RESULTS

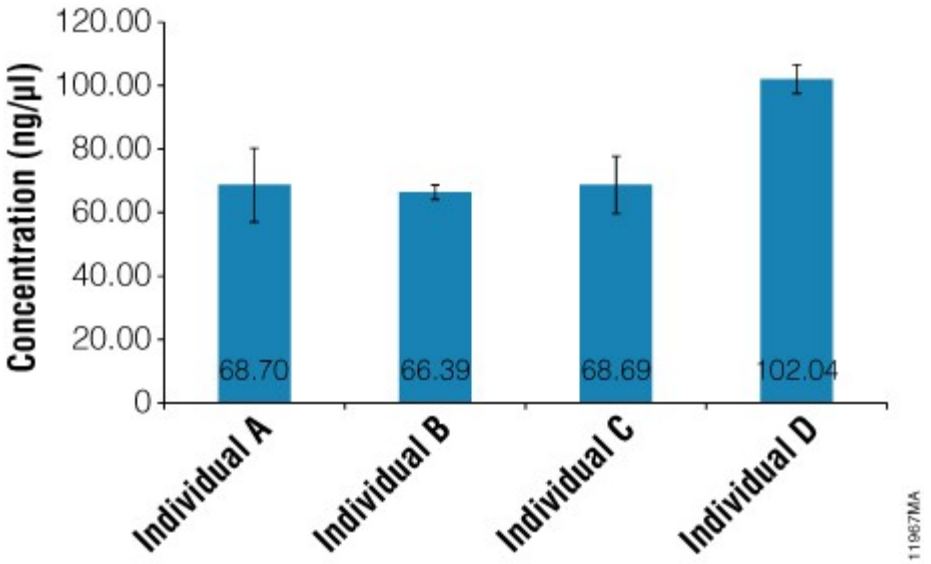

Figure 1. RNA Concentration measured by a NanoDrop® 1000. N = 3.

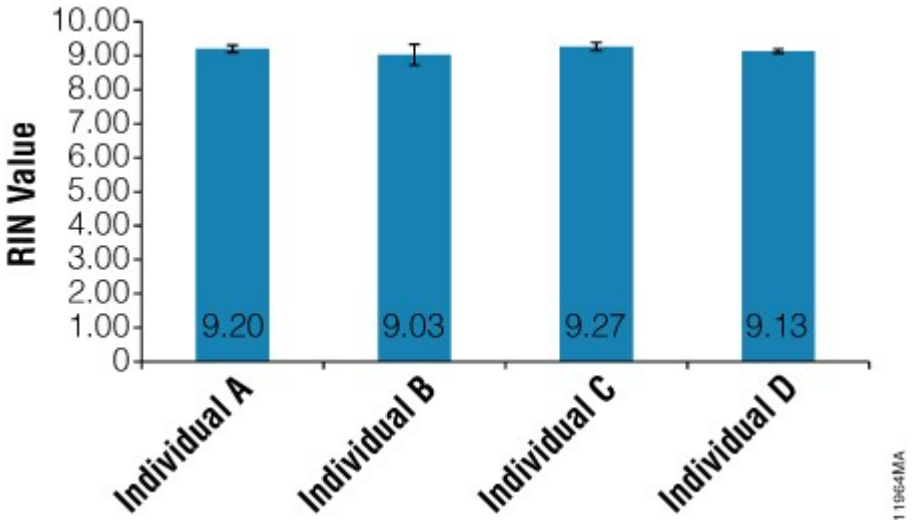

Figure 2. RIN Values were determined using an Agilent BioAnalyzer 2100 using the Agilent RNA 6000 Nano Kit and Reagents. Manufacturer's instructions were followed. N = 3.

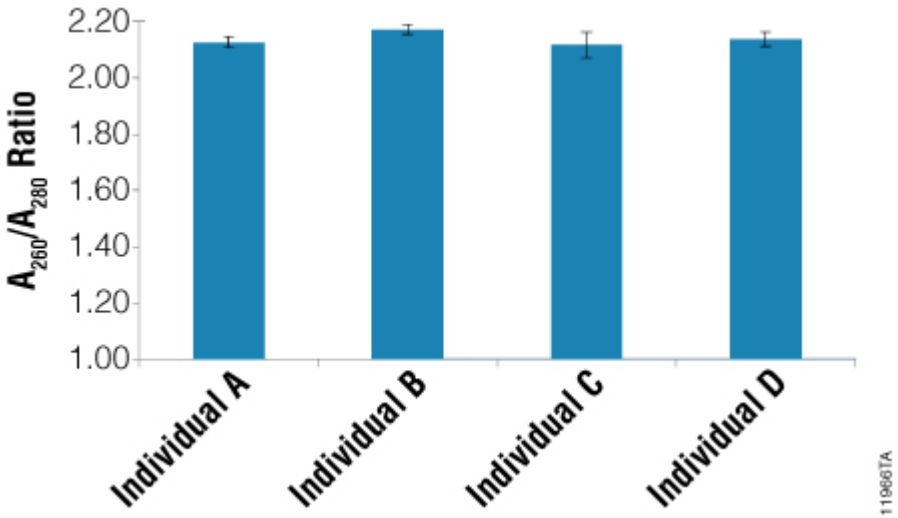

Figure 3. A_{260}/A_{280} purity ratios measured by a NanoDrop 1000. N = 3.

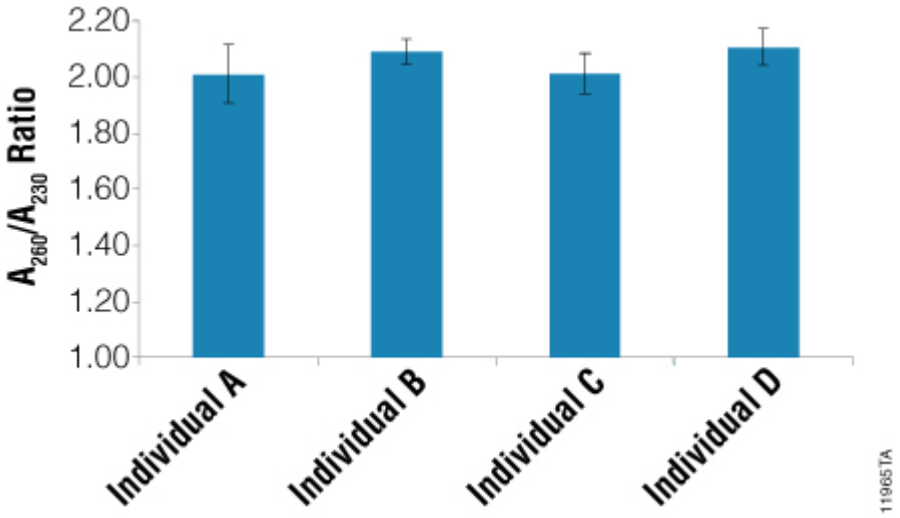

Figure 4. A_{260}/A_{230} purity ratios measured by a NanoDrop® 1000. N = 3.

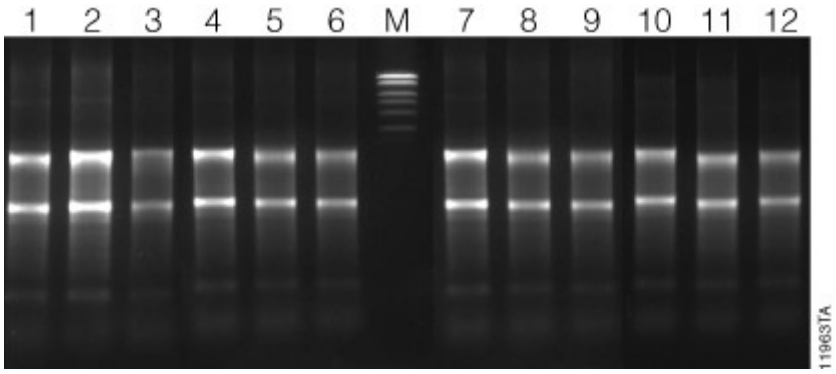

Figure 5. Agarose gel of purified RNA. A total volume of 10µl of each purified RNA sample was loaded onto a 1% agarose gel and run at 100V for 30 minutes. Lanes 1–3: Individual A; Lanes 4–6: Individual B; M = 1kb BenchTop Markers (Cat.# G7541); Lanes 7–9: Individual C; Lanes 10–12: Individual D.

SUMMARY

RNA can be successfully extracted from blood collected in BioMatrica RNAgard® tubes using the Maxwell® 16 instrument and the Maxwell® 16 LEV simplyRNA Blood Kit chemistry. Extracted RNA using the Maxwell® yielded high RIN (RNA Integrity Numbers) and good purity ratios. Furthermore RNA extracted using this chemistry and instrument showed intact ribosomal bands on a 1% agarose gel.

REFERENCES

1. Gorshe, R. and Wieczorek, D. (2011) Maxwell® 16 LEV simplyRNA Cell and Tissue Kits: A comparison to QIAcube® and TRIzol®. *Promega Corporation Website.*