Maxwell® 16 LEV simplyRNA Blood Kit: A Comparison to QIAcube® and TRIzol®

A Maxwell® 16 LEV simplyRNA Blood Kit Application Note

Rebecca Gorshe, Michelle Mandrekar, Jami English and Arune Brekke , Promega Corporation

Sample Types:
- White blood cell pellets
- Stabilized whole blood in PAXgene® tubes

Instrument Requirements:
- Maxwell® 16 Instrument (Cat.# AS2000) with firmware ≥4.9 **or** Maxwell® 16 Instrument (Cat.# AS3000) with firmware ≥1.4
- High-Strength Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070)

Maxwell® 16 LEV simplyRNA Kits:
- Maxwell® 16 LEV simplyRNA Blood Kit (Cat.# AS1310)

Performance Comparison
- QIAcube® /QIAamp® RNA Blood Mini Kit
- PreAnalytiX PAXgene® Blood RNA Kit
- TRIzol® extraction

simplyRNA Blood is simply better–purifying RNA that is superior in yield and quality to RNA purified using other systems.

Introduction

The Maxwell®16 Instrument extracts nucleic acid using paramagnetic particles, allowing optimal capture, wash, and elution of the target material. Because there are no tubes or pipetting steps involved in the automated method, there are no clogs, drips, splashing or aerosols, greatly reducing contamination risk.

The Maxwell®16 Instrument is preprogrammed with numerous purification protocols, which, combined with predispensed reagent cartridges, maximize ease-of-use and convenience. Simply add samples or lysates directly to the prefilled reagent cartridges, select the method and press Start. The reagents are optimized for specific sample types and deliver maximum yield and purity.

The Maxwell® 16 LEV simplyRNA Blood Kit is used with the Maxwell® 16 Instrument configured with the LEV High-Strength Magnetic Rod and Plunger Bar Adaptor. This RNA purification procedure is a simple method with minimal lysate handling before automated purification. The low elution volume is used to generate concentrated high-quality RNA suitable for use in downstream applications such as quantitative RT-PCR. The instrument processes up to 16 samples in about 1 hour.

The simplyRNA Blood Kit provides a simple protocol for isolating RNA from fresh whole blood drawn in an EDTA tube and a method for whole blood that has been stabilized in a PAXgene® RNA tube. Here, we compare the performance of the Maxwell® 16 LEV simplyRNA Blood Kit with automated extraction methods on the QIAcube® instrument and manual extraction using TRIzol® Reagent.

Methods

All protocols were performed following the manufacturer's instructions. All samples were purified in triplicate. For automated purification on the QIAcube® instrument, the QIAamp® RNA Blood Mini Kit was used for white blood cell pellet samples and the PreAnalytiX PAXgene® Blood RNA Kit was used for the PAXgene® samples. The maximum recommended input volumes were used to generate WBC pellets (1.5ml for QIAcube®/QIAamp®, 2.5ml for simplyRNA and TRIzol®).

Spectrophotometry: After purification, RNA concentration and purity were measured using a NanoDrop® 1000 Instrument.

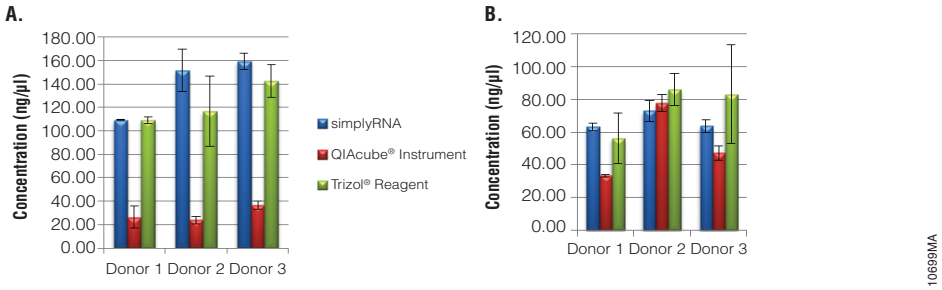

Figure 1. Concentration comparison of RNA isolated using different extraction methods. Panel A, White blood cell pellets. The maximum recommended input volumes were used to generate WBC pellets (1.5ml for QIAamp® method, 2.5ml for simplyRNA and TRIzol® methods); when corrected for input, simplyRNA Blood outperformed the QIAcube® method by 45%. Panel B, PAXgene®-stabilized blood samples. Equal volumes of blood (2.5ml) were processed for each PAXgene® sample. The QIAcube® and simplyRNA -purified PAXgene®-samples were equivalent in concentration. Data shows the mean and standard deviation for N=3 replicate samples of each type.

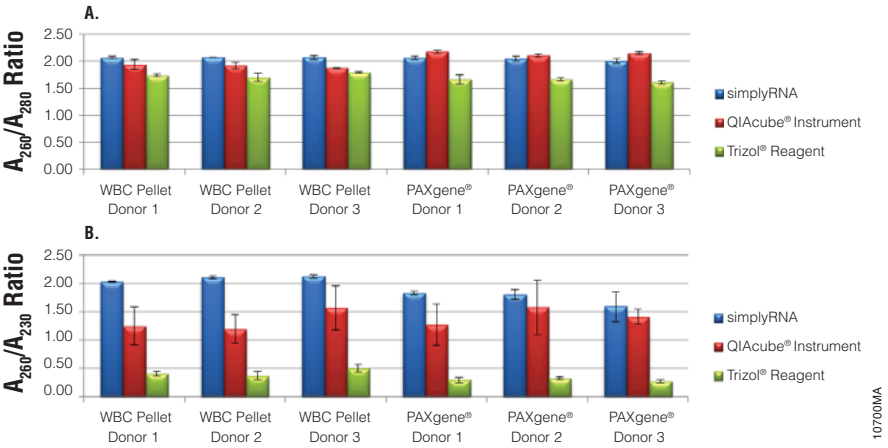

Figure 2. Purity of RNA isolated from blood using QIAcube®, simplyRNA and TRIzol® methods. Panel A, A_{260}/A_{280} ratio. Panel B, A_{260}/A_{230} ratio. Data shows mean and standard deviation for N=3 samples of each type.

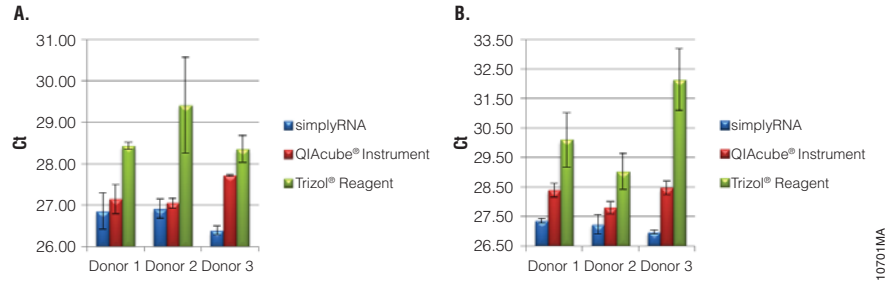

Figure 3. Ct values for RNA samples as determined by RT-qPCR. Ct values for the same amount of input RNA for each purification method are shown. Panel A, Ct Values for RNA purified from white blood cell pellets. Panel B. Ct values for samples purified from PAXgene®-stabilized blood. Data shows the mean and standard deviation from N=3 samples of each type.

Results

RNA Yield and Purity

Yield and purity of RNA isolated from white blood cell pellets or blood stored in PAXgene® tubes using all three purification methods is shown in Figures 1 and 2. Figure 1 shows a concentration comparison for each sample type. RNA samples purified with the simplyRNA Blood Kit were consistently equal to or higher in concentration than samples purified on the QIAcube® or with TRIzol® reagent. Likewise, consistent and superior purity ratios were observed with simplyRNA-purified samples, as shown in Figure 2.

RT-qPCR and gDNA Contamination

To evaluate the performance of the purified RNA in RT-PCR, we used 50ng of RNA in the GoTaq® 1-Step RT-qPCR System with RNA-specific β-2 microglobulin primers. Figure 3 demonstrates that samples purified with the simplyRNA Blood Kit gave consistently lower Ct values than QIAcube®- and TRIzol®-purified samples.

Certain downstream assays may be sensitive to any contaminating gDNA in purified RNA samples. To evaluate gDNA contamination, we used 25ng of RNA from each purification method in a Plexor® HY qPCR assay. Table 1 lists the percentage of DNA amplified per sample type. The simplyRNA Blood samples consistently showed equal or lower amounts of contaminating DNA than the samles purified with QIAcube® and TRIzol® methods.

Bioanalyzer Analysis

The Agilent 2100 Bioanalyzer was used to compare the integrity of RNA samples from each purification system. The RNA Integrity Number (RIN) for each of the RNA samples analyzed is shown in Figure 4, with a value of 10 (shown in Figure 5) being the best possible score. Overall, the RIN values for RNA purified using theMaxwell® 16 simplyRNA Blood Kit compared favorably with those for the QIAamp® Kit and the TRIzol®

methods for each sample type. The variability is likely due to varying amounts of RNases and other contaminants in blood samples. The high standard deviation in some averages is due to no RIN number being assigned for some samples in the average. This is attributed to either degraded RNA or not enough sample present to give a valid RIN number.

Conclusions

The Maxwell® 16 simplyRNA Blood Kit offers superior RNA purification compared to the QIAcube® and TRIzol® methods. Each of the comparison data pieces provide insight into why the simplyRNA Blood method is superior.

The concentration and purity data suggest that simplyRNA Blood is a more efficient system that extracts highly pure RNA. The NanoDrop® method can indicate the presence of certain contaminants, such as phenolic/guanidine carryover from TRIzol® extractions, but it is not able to differentiate between RNA and DNA. We performed qPCR analysis to evaluate the amount of gDNA carryover for each purification method. Table 1 shows that the simplyRNA Blood method is comparable to or better than TRIzol®, and superior to the QIAcube® for removal of gDNA from the samples.

RIN values demonstrate the quality and "intactness" of the extracted RNA. Lower RIN values are associated with degradation, which could indicate the presence of contaminating RNases. The RIN values for simplyRNA-purified samples are comparable or superior to those of RNA purified using the other methods.

Table 1. Percent gDNA Contamination per 25ng of RNA.

Sample Type /Purification Method	AVG(%)	SD
WBC pellet/simplyRNA Blood	0.09	0.03
WBC Pellet/QIAcube®	12.37	9.10
WBC Pellet/TRIzol®	1.90	0.38
PAXgene®/simplyRNA Blood	0.38	0.13
PAXgene®/QIAcube®	1.23	1.36
PAXgene®/TRIzol®	0.15	0.11

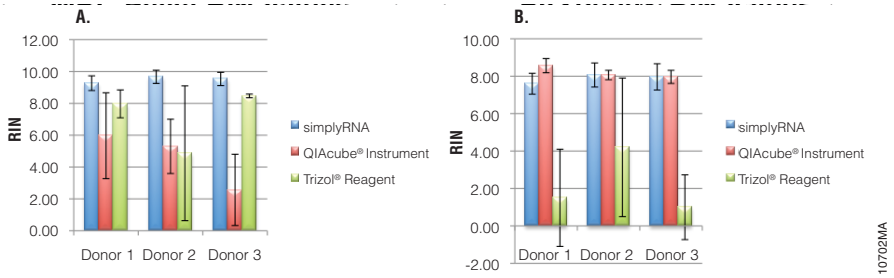

Figure 4. RIN values for RNA isolated from blood using QIAcube®, simplyRNA and TRIzol® methods. Panel A, RNA purified from white blood cells. Panel B, RNA purified from PAXgene®-stabilized blood. Standard deviations are shown for N=3 replicate samples of each type.

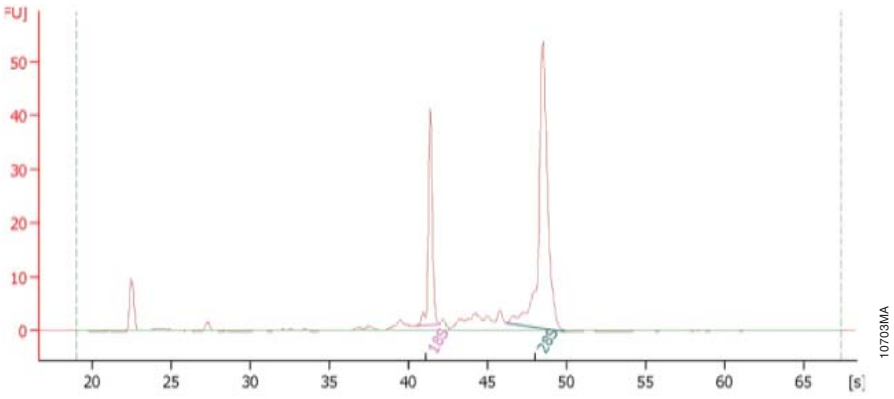

Figure 5. RIN image of RNA purified from whole blood using the simplyRNA Blood Kit. This sample had a RIN of 10.

Finally, the RT-qPCR assay results demonstrate the purity, quality, and quantity of the purified RNA. The lower Ct values of the simplyRNA-purified samples indicate that the RNA purified with this system is purer, of higher quality, and higher in concentration than RNA purified with either the QIAcube® or TRIzol® methods.

The concentration and purity data suggest that simplyRNA is a more efficient system that extracts highly pure RNA.

Ordering Information

Product	Cat.#
Maxwell® 16 Instrument*	AS2000
Maxwell® 16 MDx Instrument*	AS3000
High-Strength Magnetic Rod and Plunger Bar Adaptor	SP1070
Maxwell® 16 LEV simplyRNA Blood Kit*	AS1310
Maxwell® 16 LEV simplyRNA Cells Kit*	AS1270
Maxwell® 16 LEV simplyRNA Tissue Kit*	AS1280
Nuclease-Free Water*	P1193
Cell Lysis Solution	A7933

*For Laboratory Use.