Promega

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

A Maxwell® 16 LEV simplyRNA Tissue Kit Application Note

Materials Required

- Tissues: Mouse Brain, Heart, Liver, Kidney, Spleen
- Maxwell® 16 LEV simplyRNA Tissue Kit (Cat.# AS1280)

Instrument Requirements

- Maxwell® 16 Instrument (Cat.# AS2000) with firmware version ≥4.8 or Maxwell® 16 Instrument (Cat.# AS3000) with firmware version ≥1.3
- High-Strength Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070)

Performance Comparison

- RNeasy® Mini QIAcube® Method
- TRIzol® extraction

Introduction

The purification and analysis of targeted RNA is one of the most important techniques used to monitor the expression of genetic information within tissues. Purified RNA is routinely used in applications such as reverse transcriptase-mediated real-time PCR (RT-qPCR), cDNA synthesis and gene expression profiling such as microarray analysis in which the dynamic changes in gene expression during cellular response of natural or induced factors are monitored.

While the isolation of high-quality RNA is a critical step for the generation of meaningful experimental information, the actual process of isolating RNA can be tedious, complex and labor-intensive. Obtaining high-concentration RNA is especially important given the sensitivity and small input volume requirements of gene-expression applications. The Maxwell® 16 System was developed to meet the needs of low- to moderate-throughput users by providing automated purification of high-quality nucleic acid without considerable cost, training or maintenance. The Maxwell® 16 System extracts nucleic acid using paramagnetic particles, allowing optimal capture, wash and elution of the target material. Because there are no clogs, drips, splashing or aerosols, the contamination risk is greatly reduced. The instrument processes up to 16 samples in 30–60 minutes, depending on the sample type.

The Maxwell® 16 LEV simplyRNA Tissue Kit offers a simple protocol for isolating RNA from fresh or frozen tissue pellets. This application note compares the performance of the Maxwell® 16 LEV simplyRNA Tissue Kit with automated extraction using the RNeasy® Mini QIAcube® Kit and manual extraction using TRIzol® Reagent.

Methods

Ten, twenty or thirty milligram quantities of mouse liver, brain, heart, kidney and spleen (Pel-Freez) were homogenized using a Tissue-Tearor™ (BioSpec) according to the recommended method for each purification system. For the Maxwell® 16 LEV simplyRNA Tissue method, 200µl of Homogenization Buffer + 2% 1-thioglycerol and 200µl of Lysis Buffer were used per sample. For the RNeasy® method, 350µl (standard) or 600µl (large samples) of Buffer RLT + beta-mercaptoethanol were used per sample. For the TRIzol® method, 500µl of TRIzol® was used per sample. Maxwell® 16 LEV simplyRNA and QIAcube®-processed samples were eluted in 50µl, and TRIzol® samples were eluted in 100µl of nuclease-free water. Some characteristics of the tissues used are listed in Table 1.

Table 1. Characteristics of Tissues Processed.

Tissue Type	Characteristics and Comments
Brain	Fatty tissue; difficult to isolate RNA.
Heart	Low RNA yield.
Liver	High-yield, commonly used tissue.
Kidney	High-yield tissue.
Spleen	High-yield, but contains high levels of RNase, making extraction difficult.

RNA Concentration, Yield and Purity

Concentration, yield, and purity of the RNA samples were determined using a NanoDrop®-1000 instrument. Real-time PCR was performed using the GoTaq® 2-Step RT-qPCR System and a Bio-Rad CFX96™ Real-Time PCR instrument. RNA was reverse transcribed using random primers and ß2-microglobulin was used as the target for amplification during qPCR.

RNA samples were tested for DNA contamination using ß-actin primers in TaqMan® assays (Applied Biosystems). RNA (100ng; 5µl) was added to the TaqMan® assay in a 20µl total reaction volume. Mouse genomic DNA ranging from 100ng to 0.01ng was included as a standard to accurately quantitate the amount of DNA present in RNA samples. The Agilent 2100 Bioanalyzer (Agilent Technologies) was used to compare the integrity of RNA samples from each purification system. RNA Integrity Number (RIN) values were calculated for 1µl samples ranging from 25–500ng/µl using the Agilent RNA 6000 Nano Kit.

Results

RNA Yield and Purity

The Maxwell® 16 LEV simplyRNA Tissue Kit is designed to process 10mg of tissue. In these experiments, purification from 20mg and 30mg of tissue was evaluated to determine performance with higher amounts of tissue. In some tissues with lower RNA yield (brain, heart), an increase in yield is observed using higher amounts of starting material. With high-yield tissues (kidney, liver, spleen), we did not see proportional gains in yield across the range (Figure 1). Also, in some cases with high-yield tissues, there was a decline in yield using 30mg of starting material, which is to be expected when the concentration of RNA is very high. In all cases, the 10 and 20mg Maxwell® 16 simplyRNA Tissue samples generated comparable yields to the QIAcube® RNeasy®-purified samples. Note that the QIAcube® method has a special protocol for 30mg of tissue, as that quantity is not in the standard range of the RNeasy® kit against which we benchmarked.

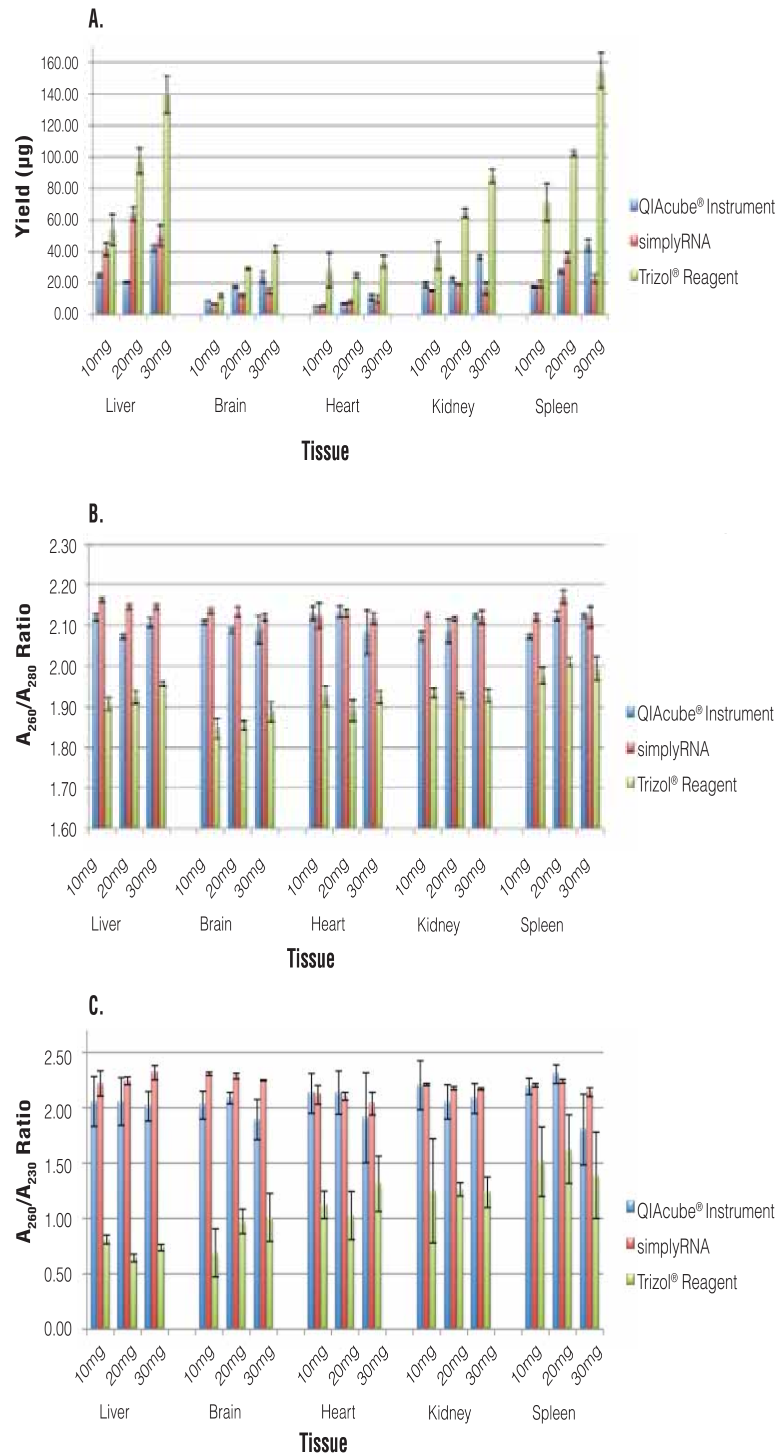

Figure 1. Yield (in µg; Panel A) and purity (Panel B and C) of RNA isolated from 10, 20 and 30mg samples of mouse liver, brain, heart, kidney and spleen tissue using RNeasy® (QIAcube®), Maxwell® 16 simplyRNA and TRIzol® methods. Data shows the mean and standard deviation for n=3 replicates per sample

RNA purity for Maxwell® 16 LEV simplyRNA tissue samples meets or exceeds A_{260}/A_{280} and A_{260}/A_{230} ratios of RNeasy® Mini QIAcube® kit-purified samples for all the amounts tested. TRIzol® showed evidence of contamination with low A_{260}/A_{280} and A_{260}/A_{230} ratios. Ratios for Maxwell® 16 LEV simplyRNA Tissue Kit-purified samples were >2.0 (Figure 1).

Amplifiability

In addition to RNA yield, most downstream applications require RNA without interfering compounds or inhibitors that may negatively affect amplification. When 100ng of RNA purified from tissue samples was used in RT-qPCR, the calculated C_t values for simplyRNA samples were equivalent to or below those obtained for RNeasy® QIAcube® samples (Figure 2). These data indicate that Maxwell® 16 simplyRNA purifies highly amplifiable RNA without the carryover of potential inhibitors that may be present in the RNeasy® QIAcube® and TRIzol® kits.

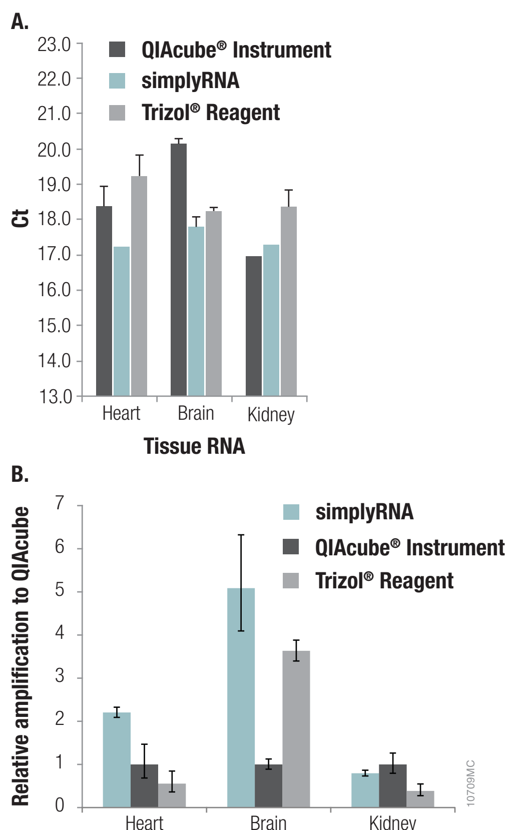

Figure 2. Amplifiable RNA for all three kits. Panel A. C_t values for normalized RNA samples as determined by GoTaq® 2-Step RT-qPCR. One hundred nanograms of RNA was used for each RT reaction. Ten microliters of the RT reaction was used for qPCR. Standard deviations are shown for n=3 reactions. **Panel B.** Relative amplifiable RNA. Data were derived from C_t values and relative amplification was determined for each kit as benchmarked to QIAcube®.

gDNA Contamination

To test for gDNA contamination C_t values were calculated from TaqMan® assays using ß-actin primers with mouse genomic DNA standards. RNA purified by Maxwell® 16 simplyRNA Tissue Kit had significantly reduced amounts of gDNA contamination compared to samples purified with the RNeasy® Mini QIAcube® kit and TRIzol® methods. The RNeasy® method showed a 7- to 14-fold increase in gDNA contamination over the simplyRNA method, while TRIzol® -purified samples showed a 12- to 94-fold increase (Table 2).

Table 2. Average Amount of DNA Present in 100ng of RNA Isolated. Standard deviations shown for n=3 samples.

Kit	Heart	Brain	Kidney
Maxwell® 16 LEV simplyRNA Tissue	0.06+0.02	0.11+0.02	0.14+0.03
RNeasy®/QIAcube®	0.44+0.10	0.80+0.08	1.99+0.58
TRIzol®	0.94+0.37	10.38+2.78	1.68+0.98

Bioanalyzer Analysis

The Agilent 2100 Bioanalyzer was used to compare the integrity of the purified RNA samples. The RIN for each sample is shown in Figure 3, with a value of 10 being the best possible score. RIN values for RNA purified by Maxwell® 16 LEV simplyRNA Tissue method varied between tissue types but compared favorably with those for the RNeasy® Mini QIAcube® Kit, and were consistently equal to or higher than those for TRIzol® Reagent.

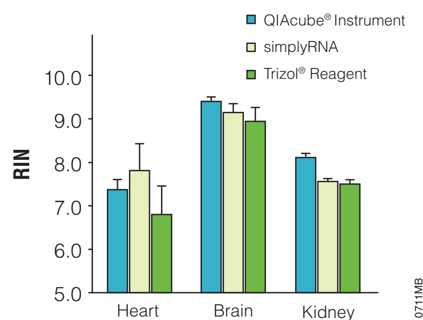

Figure 3. Average RIN for RNA samples purified by the various purification systems as determined by the Agilent 2100 Bioanalyzer. Standard deviations are shown for n=3 samples of each type.

Conclusion

Performance of the Maxwell® 16 LEV simplyRNA Tissue Kit compares well with that of the RNeasy® Mini QIAcube® kit. For difficult, low-yield tissues, the Maxwell®16 LEV simplyRNA Tissue Kit outperformed RNeasy® Mini QIAcube® and gave equivalent performance with high-yield tissues. RNA was purified successfully from a variety of tissues, was of consistently high quality and purity, and was suitable for use in a variety of downstream applications.

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