RNA Purification Kit Comparison: Yield, Quality and Real-Time RT-PCR Performance

ABSTRACT Promega offers total RNA isolation kits in a range of formats designed to meet different sample size and throughput needs. We compared the yield and quality of RNA from these kits to RNA isolated using Qiagen and Invitrogen kits. We also compared RNA performance in real-time RT-PCR. These data highlight the quality of Promega kits and utility in gene expression profiling. By comparing a breadth of kits, we found there can be kit-dependent differences in gene expression levels detected by real-time RT-PCR, and this should be considered when making quantitative RT-PCR comparisons.

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INTRODUCTION

Promega has provided high-quality total RNA isolation kits for more than 10 years. One of the most popular kits is the SV Total RNA Isolation System[(a)], a silica column-based technology for RNA isolation by spin or vacuum in a single-sample (1) or 96-well format (2). For larger sample types, we offer the PureYield™ RNA Midiprep System[(b)], which has an improved silica column format for spin or vacuum processing of as much as 300 mg of tissue, depending on the tissue type (3). Most recently our offerings have expanded to include automated RNA purification with the Maxwell® 16 Instrument. The Maxwell® 16 Instrument uses paramagnetic silica particles in prefilled reagent cartridges to isolate RNA from as many as 16 samples in 30–40 minutes (4).

Many commercially available RNA isolation kits use a similar reagent chemistry to Promega systems, and all follow a basic protocol: 1) tissue homogenization in an extraction buffer that releases RNA and inactivates RNases, 2) binding of RNA to silica in the presence of chaotropic salts, 3) washing away contaminants with alcohol-containing wash buffers, then 4) elution of the RNA with water or TE Buffer.

Although many kits are similar, we wanted to investigate how the output varied. We compared the amount and quality of RNA isolated by different silica column- and particle-based kits and compared this to a more traditional phenol-based organic extraction method (Table 1). Because one of the primary applications of RNA isolation procedures is the analysis of gene expression, we then compared RNA performance in real-time RT-PCR. In addition, data from an independent comparison made by Wyeth Pharmaceuticals is included. The RNA samples isolated using the Promega Maxwell® 16 Tissue LEV Total RNA[(c)], SV Total RNA, and PureYield™ RNA Midiprep kits were all high-yield, high-quality and gave excellent performance in real-time RT-PCR.

RNA YIELD

For these studies, we extracted RNA from frozen mouse liver following the standard tissue protocol for each kit. All protocols required tissue homogenization in a guanidinium-containing extraction buffer. Homogenization was performed on ice with a Tissue-Tearor™ Homogenizer (BioSpec Products, Inc.). Extractions were done in replicates of six for the automated systems (Maxwell® 16 and Qiagen EZ1™) and in triplicate for the manual systems. Ten milligrams of tissue was used per extraction, except for PureYield™ RNA Midiprep System and Invitrogen TRIzol® Reagent (70 mg/extraction).

Table 1. RNA Purification Kits Compared in this Study.

Purification Kit Name	Format	Mechanism of RNA Purification
Maxwell® 16 Tissue LEV Total RNA Kit (Cat.# AS1220)	Automated (16 samples/run)	Paramagnetic Silica Particles
EZ1™ RNA Tissue Mini (Qiagen)	Automated (6 samples/run)	Paramagnetic Silica Particles
SV Total RNA Isolation System (Cat.# Z3100)	Manual	Silica Membrane
PureYield™ RNA Midiprep System (Cat.# Z3740)	Manual	Silica Membrane
RNeasy® Mini Kit (Qiagen)	Manual	Silica Membrane
TRIzol® Reagent (Invitrogen)	Manual	Organic Extraction

Yield was evaluated by absorbance at 260 nm. For easy comparison, the data are expressed as yield per 10 mg of tissue in Figure 1. Organic extraction with Invitrogen TRIzol® Reagent gave the highest RNA yield at 62 µg/10 mg of tissue. For the silica-based chemistries, the highest RNA yields were obtained using Maxwell® 16 LEV and SV Total RNA, followed by PureYield™ RNA Midiprep. The lowest yields per tissue mass were obtained with the Qiagen kits. The RNA isolated in these experiments then was used for all subsequent testing.

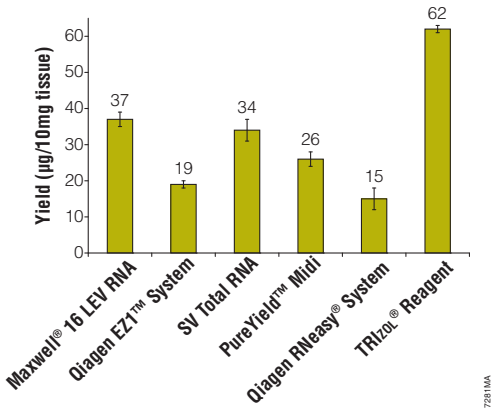

Figure 1. Comparison of RNA yield from mouse liver. RNA was isolated from frozen mouse liver samples following the standard protocol for each kit. Ten milligrams of tissue was processed for all kits except PureYield™ RNA Midiprep and TRIzol® Reagent (70 mg/extraction). Yield was determined by absorbance at 260 nm using the NanoDrop® ND-1000 spectrophotometer and expressed as yield per 10 mg of tissue to standardize for differences in input between kits. Data are the average ± standard deviation of triplicate experiments, except Maxwell® 16 and Qiagen EZ1™ systems (n = 6).

RNA QUALITY

Suitability of RNA for downstream applications is typically assessed by absorbance. Absorbance can detect contaminants in the RNA: 280 nm for protein and 230 nm for contaminants such as phenols and guanidine isothiocyanate commonly used in RNA extractions. For high-quality RNA, absorbance at both wavelengths should be about half the value of the 260 nm absorbance to give a ratio ~2. Table 2 gives the average A_{260}/A_{280} and A_{260}/A_{230} ratios. All kits yielded RNA with both ratios at or greater than 2.0, except the Qiagen EZ1™ (A_{260}/A_{230} = 1.88).

RNA integrity can be assessed qualitatively by gel or quantitatively using systems such as the Agilent Bioanalyzer, which uses microfluidics to size-separate and quantitate RNA. The Bioanalyzer measures the amount of 28S and 18S ribosomal RNA; high-integrity RNA has a 28S:18S ratio of ~2.0. The Bioanalyzer also calculates an RNA Integrity Number (RIN), which considers the full size distribution of RNA, not just the 28S and 18S rRNA, and is considered a more accurate assessment of overall integrity (5). High-integrity RNA has a RIN value greater than 8.0. Table 2 gives the 28S:18S ratio and RIN for RNA from the silica-based extractions. The 28S:18S ratios for PureYield™ RNA Midiprep were superior to all other kits tested; however, all kits had high RIN values (≥9.0) except the Qiagen EZ1™ System (RIN = 6.4). The low RIN indicates the Qiagen EZ1™ samples were partially degraded; this is supported by the Bioanalyzer electrophoretic trace (Figure 2).

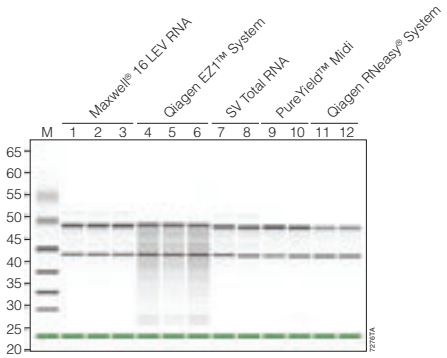

Figure 2. Electrophoretic trace of RNA. To qualitatively assess RNA integrity, 2 or 3 RNA samples from each silica-based kit were diluted in water to roughly equivalent concentrations, then analyzed using the Agilent 2100 Bioanalyzer with an RNA 6000 Nano LabChip®.

GENOMIC DNA

The presence of genomic DNA can be problematic in gene expression studies, as it can lead to an overestimation of transcript quantity as well as false-positive results by detecting DNA during RT-PCR. For this reason, RNA isolation procedures often include a strategy to minimize the co-isolation of genomic DNA. The kits tested here use several different strategies: DNA Clearing Agent (Maxwell® 16 and PureYield™ Systems), DNase treatment (SV Total RNA, EZ1™ and RNeasy® Systems) or

Highest RNA yields for the silica-based chemistries were obtained using the Maxwell® 16, SV Total RNA and PureYield™ Midiprep Kits.

The low RIN indicates that the Qiagen EZ1™ samples were partially degraded.

Table 2. Comparison of RNA Quality. RNA purity was determined using the ratio of absorbance at 260 nm/280 nm or 260 nm/230 nm. To evaluate RNA integrity, 2 or 3 RNA samples from each kit were diluted in water to roughly equivalent concentrations, then analyzed using the Agilent 2100 Bioanalyzer with an RNA 6000 Nano LabChip®. Ratio of 28S:18S rRNA and RIN measurements are the average ± standard deviation (n = 2 or 3). Integrity was examined only in the silica-based extractions.

Isolation Method	A_{260}/A_{280}	A_{260}/A_{230}	rRNA Ratio [28S/18S]	RNA Integrity Number (RIN)
Maxwell® 16	2.14 ± 0.02	2.12 ± 0.01	1.6 ± 0.1	9.4 ± 0.1
Qiagen EZ1™	2.06 ± 0.01	1.88 ± 0.01	1.0 ± 0.1	6.4 ± 0.3
SV Total RNA	2.19 ± 0.02	2.35 ± 0.05	1.5 ± 0.0	9.0 ± 0.0
PureYield™ Midi	2.19 ± 0.02	2.07 ± 0.14	1.9 ± 0.2	9.6 ± 0.1
Qiagen RNeasy® Kit	2.14 ± 0.14	2.37 ± 0.14	1.1 ± 0.1	9.3 ± 0.0
TRIzol® Reagent	2.01 ± 0.01	2.10 ± 0.17	–	–

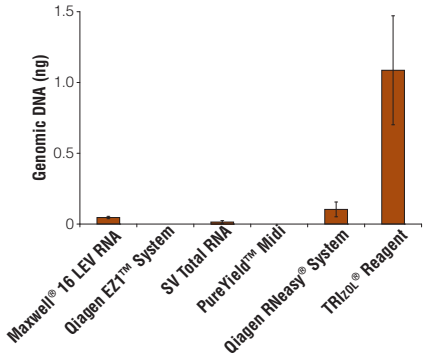

Figure 3. Genomic DNA detected in 100 ng RNA. Genomic DNA in RNA isolated by each indicated kit was quantitated by real-time PCR detection of β-actin without an initial reverse transcription step using the Applied Biosystems TaqMan® Assay. RNA (100 ng) was analyzed per reaction, and the C_t value was compared to the C_t value of serially diluted mouse genomic DNA to calculate the absolute amount of DNA present. Data are the average ± standard deviation of three samples for each purification kit analyzed in duplicate. Samples with no detectable DNA had less than the minimum detectable standard (0.01 ng).

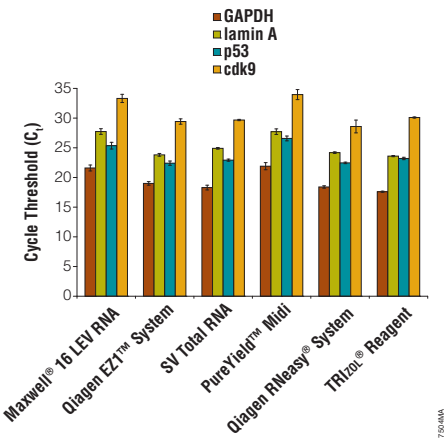

Figure 4. Detection of high-, medium-, and low-abundance transcripts by TaqMan® real-time PCR. RNA isolated in the experiment described in Figure 1 was analyzed by the Applied Biosystems TaqMan® real-time RT-PCR assays for the indicated transcripts on the BioRad Chromo4™ Real-Time PCR Detection System. Data are the average ± standard deviation of duplicate reactions (n = 3–6).

phase partitioning (TRIzol® reagent). To assess genomic DNA removal efficiency, we measured the amount of detectable β-actin DNA using Applied Biosystems TaqMan® real-time PCR. Amplification was carried out with 100 ng of RNA without an initial reverse transcription step. DNA was quantitated by comparing the C_t values to those of serially diluted Mouse Genomic DNA (Cat.# G3091). All methods except TRIzol® reagent were relatively efficient at minimizing genomic DNA co-isolation with the RNA (≤0.1 ng/100 ng input RNA). No DNA was detected above background (<0.01 ng DNA) in RNA isolated by PureYield™ RNA Midiprep or Qiagen EZ1™ Systems.

High-, medium- and low-abundance transcripts were detectable regardless of the RNA purification method used.

GENE EXPRESSION

RNA transcripts are present at a wide range of concentrations within cells. Often highly abundant RNAs, such as transcripts from housekeeping genes like GAPDH or β-actin, are easily detectable, whereas less abundant transcripts can be more difficult to detect. Using TaqMan® real-time RT-PCR assays, we tested the ability to detect high- (GAPDH), medium- (lamin A and p53), and low- (cdk9) abundance transcripts in each of the RNA preparations. All transcripts were detected in 100 ng of RNA regardless of the RNA purification method used (Figure 4). All transcripts also could be detected in 10 ng of RNA, although the C_t values for the less abundant cdk9 transcript was ≥35 for all of the silica-based purification kits, which is arguably outside the reliable range of detection (data not shown).

The average transcript C_t value varied by 3–4 cycles between purification kits, with RNA from Maxwell® 16 and PureYield™ RNA Midiprep giving higher C_t values than the rest. This variation was not due to contaminants in the purified RNA, since adding each RNA to an independent control TaqMan® RT-PCR did not affect control detection, even when the RNA was added in excess (1 µg of RNA, data not shown). We also compared the C_t values for each transcript in Figure 4 at 10 ng and 100 ng input RNA; all kits resulted in a C_t value change of ~3.3, as expected for a 10-fold difference in input (data not shown). This indicates the high purity of all RNA tested and the absence of contaminants that may affect real-time PCR analysis.

TRANSCRIPT QUANTITATION

Real-time RT-PCR is used in gene expression studies to quantitate transcripts. Transcript quantitation can be absolute, by comparing C_t values to a standard curve, or relative, by comparing C_t values to the C_t value of a second transcript present at consistent levels (e.g., housekeeping gene transcripts). The 3- to 4-cycle difference between transcripts isolated by different kits indicates absolute quantitation results could be affected by 8- to 16-fold (fold difference = $2\Delta C^t$).

By comparing the relative ratio of each transcript's C_t value to that of GAPDH, we estimated whether relative quantitation results also could be affected by the purification method (Figure 5). The ratios for each transcript varied by only 10%, with RNA from SV Total RNA and TRIzol® showing the greatest deviation. RNA from Maxwell® 16 and PureYield™ RNA Midiprep, the kits that gave the highest C_t values, gave equivalent relative ratios to the Qiagen kits. This suggests relative quantitation is less likely to be impacted by the RNA purification method used. While each kit isolates the total pool of messenger RNA, differences in absolute C_t values could be due to variation in the amount of other RNA

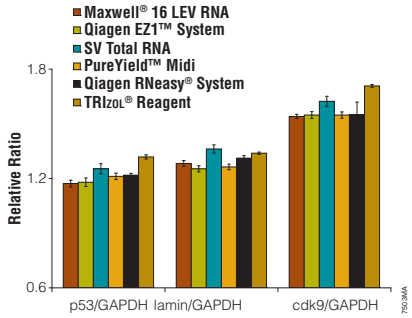

Figure 5. Relative ratios of each transcript to GAPDH showing only slight variations for all RNA tested. Using the data for 100 ng cDNA shown in Figure 4, relative transcript levels were determined. C_t values for lamin, p53 and cdk9 were divided by the C_t value for GAPDH in each sample. Ratios then were averaged for each purification chemistry and transcript. Data are the average ± standard deviation of 3–6 purifications analyzed by TaqMan® in duplicate.

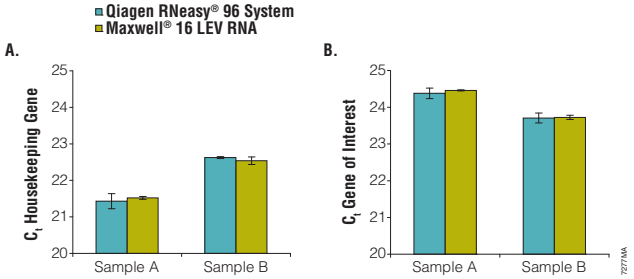

Figure 6. Comparison of RNA isolated with the Maxwell® 16 and Qiagen RNeasy® 96 kits in quantitative real-time PCR. RNA was isolated from mesenchymal cells using the Maxwell® 16 Cell LEV RNA kit and Qiagen RNeasy® 96 kit. Equivalent amounts of RNA from each kit were analyzed by TaqMan® Assay for a housekeeping gene transcript (A) and the transcript of a gene of interest (B). Data are the average ± standard deviation of three purifications.

(i.e., transfer RNA, ribosomal RNA) isolated. Messenger RNA comprises only ~2–3% of the total RNA in mammalian cells, so even a slight difference in a kit's efficiency at isolating nonmessenger RNA could have a significant impact on mRNA as a percent of the total RNA isolated.

WYETH PHARMACEUTICALS STUDY

Wyeth Pharmaceuticals compared RNA isolated with Maxwell® 16 Cell LEV RNA Kit[c] to their current method of RNA isolation. RNA was extracted from human mesenchymal stem cells. RNA purity was measured by absorbance, and performance was evaluated by real-time RT-PCR. Both extraction methods gave pure RNA as determined by A_{260}/A_{280} absorbance ratios (Maxwell® 16 and current method, 1.8 ± 0.1 and 1.9 ± 0.1, respectively). The RNA also yielded highly consistent C_t values for two different transcripts and both sample groups tested (Figure 6).

SUMMARY

This study compares the yield, quality, and real-time PCR performance of RNA isolated by Promega, Qiagen and Invitrogen kits. From the single, small-scale prep of the SV Total RNA Isolation System to the medium-scale PureYield™ RNA Midiprep to the automated Maxwell® 16 LEV Total RNA Purification Kit, the Promega kits gave high yield and high-quality RNA that performed well in TaqMan® real-time RT-PCR assays and RNA integrity using the Bioanalyzer. Variation in transcript C_t values suggests relative, rather than absolute, quantitation should be used to compare quantitative RT-PCR results from RNA isolated by different kits.

REFERENCES

1. Brisco, P. *et al.* (1997) *Promega Notes* **64**, 7–12.
2. Grunst, T. (2001) *Promega Notes* **79**, 29–32.
3. Brisco, P. *et al.* (2006) *Promega Notes* **92**, 14–9.
4. Koller, S. *et al.* (2007) *Promega Notes* **97**, 9–11.
5. Mueller, O. *et al.* (2004) RNA integrity number (RIN) standardization of RNA quality control. Agilent Technologies.

PROTOCOLS

- *Maxwell® 16 Tissue LEV Total RNA Purification Kit Technical Bulletin* #TB367, Promega Corporation **www.promega.com/tbs/tb367/tb367.html**
- *Maxwell® 16 Cell LEV Total RNA Purification Kit Technical Bulletin* #TB368, Promega Corporation **www.promega.com/tbs/tb368/tb368.html**
- *SV Total RNA Isolation System Technical Manual* #TM048, Promega Corporation **www.promega.com/tbs/tm048/tm048.html**
- *PureYield™ RNA Midiprep System Technical Manual* #TM279, Promega Corporation **www.promega.com/tbs/tm279/tm279.html**

ORDERING INFORMATION

Product	Size	Cat.#
Maxwell® 16 Tissue LEV Total RNA Purification Kit	48 preps	AS1220
Maxwell® 16 Cell LEV Total RNA Purification Kit	48 preps	AS1225
SV Total RNA Isolation System	50 preps	Z3100
PureYield™ RNA Midiprep System	10 preps	Z3740

For Laboratory Use.

Promega kits gave high yield and high-quality RNA that performed well in TaqMan® real-time PCR assays.

[a]Australian Pat. No. 730718 and other patents and patents pending.

[b]Patent Pending.

[c]U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, Australian Pat. No. 732756 and other patents and patents pending.

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