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

A Maxwell® 16 LEV simplyRNA Cells Kit Application Note

**Materials Required**

- Cell types: Jurkat, HEK293, 3T3 cells
- Maxwell® 16 LEV simplyRNA Cells Kit (Cat.# AS1270)

**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 cells. 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 processed.

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

## Methods

$10^6$, $10^5$, $10^4$, and $10^3$ HEK293 (human), Jurkat (human) and 3T3 (mouse) cells were dispensed into microcentrifuge tubes and harvested by centrifugation. The media was removed and the resulting pellets stored at –80°C until use. Cells were processed according to the recommended method for each purification system. For the simplyRNA method, 200µl of Homogenization Buffer + 2% 1-thioglycerol and 200µl of Lysis Buffer were added per sample. For the RNeasy® method, 350µl of Buffer RLT + beta-mercaptoethanol was added per sample, and each sample was passed through a 20-gauge needle. For the TRIzol® method, 500µl of TRIzol® was added per sample and each sample was passed through a 20-gauge needle. All samples were eluted in 50µl of nuclease-free water.

### Cells Processed

A summary of the cells processed, including characteristics and typical RNA yields, is provided in Table 1.

Table 1. Characteristics of Cells Processed.

| Cell Type | Characteristics and Comments |
|---|---|
| HEK293 | Human embryonic kidney cells; adherent, medium to high total RNA yield |
| Jurkat | Human T cell line; nonadherent (grows in suspension), typically low RNA yield |
| 3T3 | Mouse fibroblast cell line; adherent, medium to high RNA yield |

### RNA Concentration, Yield and Purity

Concentration, yield and purity of the RNA samples were determined using a NanoDrop®-1000 instrument. Quantitative PCR was performed using the GoTaq® 2-Step RT-qPCR System and a CFX96™ Real-Time PCR instrument (Bio-Rad, Inc.). 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 (mouse) or CAPZA3 (human) primers in TaqMan® assays (Applied Biosystems). RNA (100ng; 5µl) was added to the TaqMan® assay in a 20µl total reaction volume. Mouse or human 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:** RNA yields from cells using the Maxwell® 16 LEVsimplyRNA Cells Kit were comparable to those obtained using the QIAcube® RNeasy® method. This is demonstrated in the NanoDrop® ($10^6$ and $10^5$ cells; Figure 1) data. RNA extracted using TRIzol® was at or above the values obtained for the other two kits. However, when looking at RNA purity, contamination in the Trizol® samples (as evidenced by the low $A_{260}/A_{280}$ and $A_{260}/A_{230}$ ratios) suggests that the actual total RNA yield might be much lower than that calculated by absorbance readings. Purity for the Maxwell® simplyRNA and QIAcube® methods was comparable with $A_{260}/A_{280}$ and $A_{260}/A_{230}$ ratios for $10^6$ cells >2.0 (Figure 1). Yield and purity of RNA isolated from $10^4$ and $10^3$

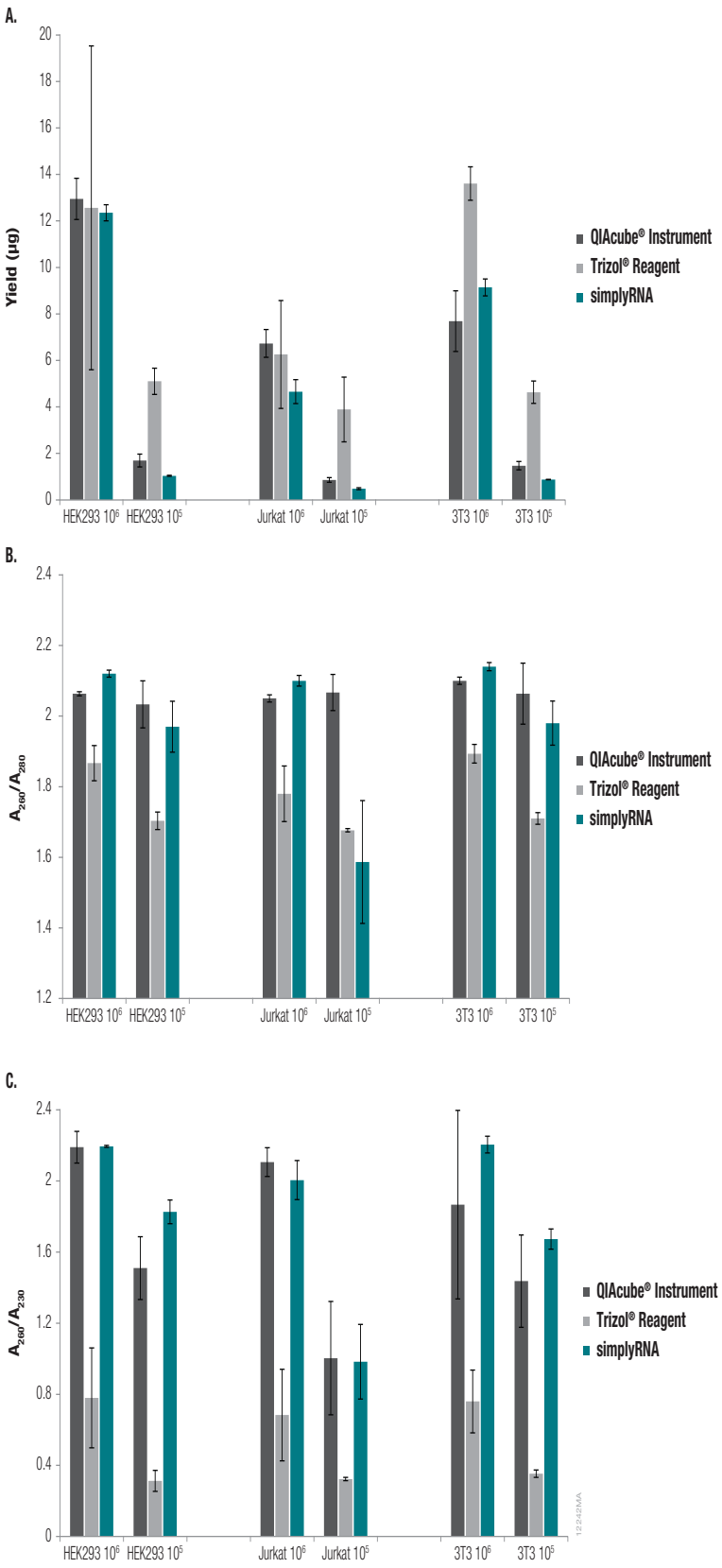


**Figure 1. RNA Yield (in µg; Panel A) and purity (Panel B and C) from from $10^6$ and $10^5$ cells using simplyRNA, RNeasy® (QIAcube®) and TRIzol® methods.** Data shows mean and standard deviation for n=3 replicates of each cell type.

## Results (continued)

cells could not be determined by NanoDrop® as the minimum detection limit is approximately 2ng/µl.

**Amplifiability:** In addition to RNA yield, most downstream applications require RNA without interfering compounds or inhibitors that may negatively affect amplification. The results from the RT-qPCR of 100ng of normalized RNA samples showed the relative ability of each kit to produce amplifiable RNA. Maxwell® 16 LEV simplyRNA Cells Kit generated lower average $C_t$ values and therefore more amplifiable RNA compared to the QIAcube® RNeasy® and TRIzol® methods (Figure 2).

**gDNA Contamination:** Genomic DNA (gDNA) contamination is an inherent problem during RNA purification that can lead to nonspecific amplification and aberrant results in many downstream assays, including RT-qPCR. To test for gDNA contamination $C_t$ values were calculated from TaqMan® assays using β-actin (mouse) or CAPZA3 (human) primers with genomic DNA standards. The results suggested that RNA purified with QIAcube® and TRIzol® Reagent methods had higher DNA contamination than the Maxwell® simplyRNA purified samples (Table 2). The QIAcube® RNeasy® method showed a 134- to 281-fold increase in gDNA contamination over Maxwell® 16 simplyRNA, and TRIzol® showed a 15- to 67-fold increase.

**Table 2. Average gDNA Contamination Present in 100ng of RNA.**
Standard deviations calculated for n=3 samples.

| Kit | HEK293 | Jurkat | 3T3 |
|---|---|---|---|
| Maxwell® 16 LEV simplyRNA | 0.03 ± 0.00 | 0.11 ± 0.02 | 0.10 ± 0.04 |
| RNeasy®/QIAcube® | 8.45 ± 2.32 | 28.53 ± 5.86 | 13.40 ± 1.00 |
| TRIzol® | 2.03 ± 1.08 | 3.94 ± 0.39 | 1.50 ± 0.08 |

**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 simplyRNA compared favorably with those for the QIAcube® RNeasy® Kit and were consistently equal to or higher than those for TRIzol® Reagent. RIN values for RNA purified from cells using Maxwell® 16 LEV simplyRNA Cells Kit were consistently at or near 10.

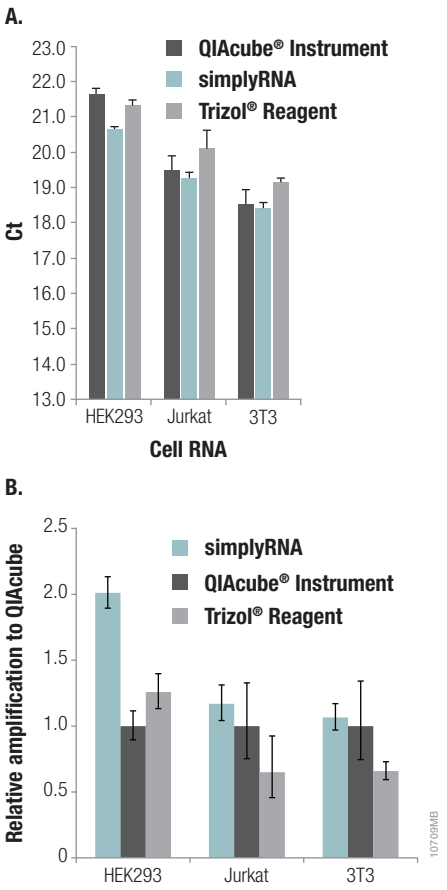


**Figure 2. Amplifiable RNA from all three kits. Panel A.** $C_t$ values for normalized RNA samples as determined by GoTaq® 2-Step RT-qPCR. One hundred nanograms of RNA was used for each RT reaction. Ten microliters of the RT reaction was used for qPCR. Standard deviations are shown for n=3 reactions.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


**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

The RNeasy® Mini QIAcube®, TRIzol® and Maxwell®16 LEV simplyRNA Cells Kit purified RNA within an acceptable yield range, as measured by absorbance quantitation. However, when comparing RNA quality, the Maxwell® 16 LEV simplyRNA Cells Kit purified RNA from cells that was highly pure, amplifiable and contained little detectable gDNA. These criteria distinguish the Maxwell® 16 LEV simplyRNA Cells Kit as a superior tool for purification of RNA that is suitable for use in a variety of downstream applications.



PROMEGA CORPORATION • 2800 WOODS HOLLOW ROAD • MADISON, WI 53711-5399 USA • TELEPHONE 608-274-4330
**www.promega.com** • ©2014 PROMEGA CORPORATION • ALL RIGHTS RESERVED • PRICES AND SPECIFICATIONS SUBJECT TO CHANGE WITHOUT PRIOR NOTICE • PRINTED IN USA 4/14 • 13061649 • PART #AN265