Maxwell® 16 Tissue and Cell Total RNA LEV Systems

ABSTRACT The Maxwell® 16 Integrated System combines compact instrumentation, optimized automated methods, prefilled reagent cartridges, service and support to save time, enhance productivity and improve the consistency of results. We have developed low-elution volume (LEV) Maxwell® 16 Total RNA Purification Kits for cells and mammalian tissues to improve convenience and enhance purified RNA performance in qRT-PCR, RT-PCR and cDNA synthesis applications. In this article we demonstrate the use of the Maxwell® 16 LEV Integrated System for total RNA purification.

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The Maxwell® 16 LEV Systems purify high-concentration total RNA (>100 ng/µl) from tissue and cultured cells.

INTRODUCTION

The purification and analysis of targeted RNA is an important technique used to monitor the expression of genetic information. Purified RNA is routinely used as the template in quantitative RT-PCR (qRT-PCR), RT-PCR or cDNA synthesis to monitor dynamic changes in gene expression due to natural or induced challenges. While isolating high-quality RNA provides the foundation for generating experimental results, obtaining high-concentration RNA is especially important given the sensitivity and small input volume requirements of gene-expression applications.

The Maxwell® 16 System is developed for low- to moderate-throughput users and provides automated purification at a scale more appropriate to their workload without considerable capital investment, training or maintenance (1). We have developed low elution volume (LEV) Maxwell® 16 Total RNA Purification Kits for cultured cells and tissues. These LEV Kits provide high-concentration RNA (greater than 100 ng/µl, depending on sample type) for enhanced performance in qRT-PCR, RT-PCR and cDNA synthesis applications. The purified RNA is of high quality, as determined by a variety of measures, and exhibits almost undetectable amounts of contaminating genomic DNA (gDNA).

The Maxwell® 16 Cell LEV Total RNA Purification Kit[a] (Cat.# AS1225) and Tissue LEV Total RNA Purification Kit[a] (Cat.# AS1220) use prefilled LEV reagent cartridges to purify ready-to-use RNA. Existing Maxwell® 16 Instruments can be upgraded using the LEV Conversion Kit for Maxwell® 16 (Cat.# AS1250) with the downloaded firmware method updates, available free of charge at: **www.promega.com/maxwell16/firmware**. In this article, we describe the key features of the Maxwell® 16 LEV Total RNA Purification Kits and provide performance data for applications such as qRT-PCR.

TOTAL RNA FROM CULTURED CELLS

The Maxwell® 16 Cell LEV Total RNA Purification Kit purifies total RNA from 1×10^4 to 2×10^6 adherent or suspension cultured cells. The kit uses a proprietary "Cytosol" buffer that gently permeabilizes the cell membrane to separate the cytoplasmic RNA from nuclear contents including gDNA.

The elution volume can be adjusted from 30 to 100 µl without affecting RNA quality. For the highest RNA concentrations, >100 ng/µl in most cell types, a 30 µl elution volume should be used. For the maximum total yield of RNA, but with a lower concentration, a 100 µl elution volume can be used (Figure 1). Total RNA yield depends on cell type, culture conditions and growth stage at the time of harvesting.

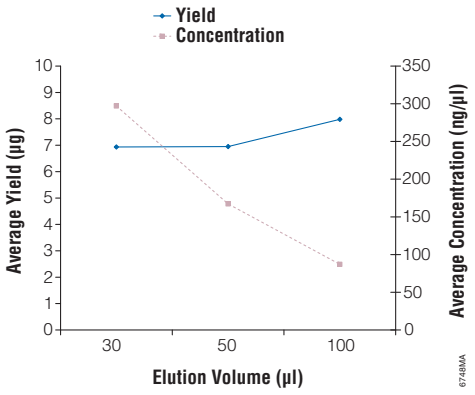

Figure 1. Effect of different elution volumes on purified total RNA yield and concentration. Total RNA was purified from 1×10^6 HEK 293 cells and eluted in 30, 50 or 100 µl of Nuclease-Free Water. The Average Yield (µg) of purified total RNA is shown as a solid line. The Average Concentration (ng/µl) of purified total RNA is shown as a dotted line. Both Yield and Concentration are plotted as a function of the elution volume used.

Integrity of RNA purified with the Maxwell® 16 LEV Systems is very high, as indicated by RIN numbers >8.0.

TOTAL RNA FROM TISSUE SAMPLES

The Maxwell® 16 Tissue LEV Total RNA Purification Kit uses the same LEV format as the Maxwell® 16 Cell LEV Total RNA Purification Kit, with the addition of a novel Clearing Agent and Spin Column that removes contaminating DNA from samples prior to RNA purification. High-concentration total RNA (>500 ng/µl) can be obtained from 10 mg of mouse tissue; however, a range of 5–25 mg input tissue can be used (Table 1). Similar results have been obtained with liver, spleen, brain, heart and lung.

The input elution volume can be adjusted from 30 to 100 µl without affecting RNA quality. Tissue with high RNA content such as mouse liver may result in decreased yield when over 20 mg is used (Table 1). Tissues with lower RNA content are less likely to be affected. For most consistent yield and concentration, 50 µl of input elution buffer is recommended.

Table 1. Titration of Mouse Liver Tissue Showing RNA Yield, Concentration and Purity. Total RNA was purified from frozen mouse liver using the Maxwell® 16 Tissue LEV Total RNA Purification Kit. Total RNA concentration and purity were measured using a NanoDrop® ND-1000 spectrophotometer. Total RNA yield was determined by multiplying the total RNA concentration by the recovered eluate volume.

Sample Size	Average Yield (µg; n = 6)	Average Concentration (ng/µl)	Average Purity (A_{260}/A_{280})	Average Purity (A_{260}/A_{230})
5 mg	11.9	580.4	2.1	2.1
10 mg	19.1	820.5	2.1	2.2
15 mg	24.4	1033.3	2.1	2.2
20 mg	28.7	1191.3	2.1	2.2
25 mg	17.4	822.7	2.1	2.1

NO DETECTABLE CROSS-CONTAMINATION

One common concern with automated systems is the possibility of cross-contamination between samples. We performed a test for cross-contamination by loading eight reagent cartridges with lysate from 20 mg of mouse liver and eight reagent cartridges with water in an alternating pattern. Samples were eluted in 50 µl of Nuclease-Free Water and assayed by qRT-PCR for mouse β-actin RNA using 5 µl of the eluate (Figure 2). None of the water controls showed quantifiable RNA (red), while the RNA purified from 20 mg of liver showed low C_t numbers (green), indicating strong amplification (Figure 2, Panel A). A further verification comes from comparing the melting points of the qRT-PCR products to those of the expected target (Figure 2, Panel B). The red melting curve (bottom of figure) with a lower temperature indicates nonspecific primer-dimer amplification. Thus, there is no detectable RNA cross-contamination between samples purified using the Maxwell® 16 Tissue LEV Total RNA Purification Kit, as assayed by qRT-PCR. A qPCR test for GAPDH genomic DNA using an intron of the GAPDH gene was also performed (data not shown).

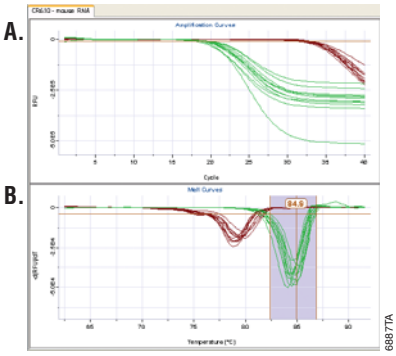

Figure 2. Plexor® qRT-PCR cross-contamination test. Total RNA was isolated from 20 mg of mouse liver (odd wells), alternating with Nuclease-Free Water (even wells), using the Maxwell® 16 Tissue LEV Total RNA Purification Kit (n = 8). Each eluate (5 µl) was assayed using FAM-labeled mouse β-actin primers and the Plexor® One-Step qRT-PCR System. The Plexor® reactions were run on an Applied Biosystems 7500 real-time PCR system, as described in Technical Manual #TM265. Data analysis was performed using the Plexor® Analysis Software. **Panel A.** Amplification curves of total RNA and water controls. The horizontal line at the top of the graph shows the calculated baseline. **Panel B.** Dissociation/melt curves of the amplification products. The expected melt temperature range of the mouse β-actin amplification product is indicated by the shaded region. The expected target melt temperature is indicated by a vertical line. RFU = relative fluorescence units.

SUPERIOR RNA QUALITY

Visualization of RNA on ethidium bromide-stained agarose gels is often used to determine RNA quality by observation of 28S and 18S ribosomal RNA bands. However, visualization is not always sensitive enough to detect slight RNA degradation (2).

RNA integrity is more accurately measured by the RNA integrity number (RIN), which is based on data from the Agilent 2100 bioanalyzer (Figure 3). The RIN takes into account the entire electrophoretic trace of the RNA including degradation products, not just the 28S:18S ribosomal RNA (rRNA) ratio. The 28S rRNA peak generally degrades before the 18S rRNA peak, and a low broad band would show the degraded products. RNA purified using the Maxwell® 16 Cell or Tissue LEV Total RNA Purification Kits and analyzed by the Agilent 2100 bioanalyzer showed two large peaks for the rRNA and had RIN values of >8.0, a measure of the highest RNA integrity. Very low amounts of DNA (<50 copies/100 ng RNA) are present. RNA obtained using both the Cell and Tissue Maxwell® 16 LEV Total RNA Purification Kits is consistently of high quality.

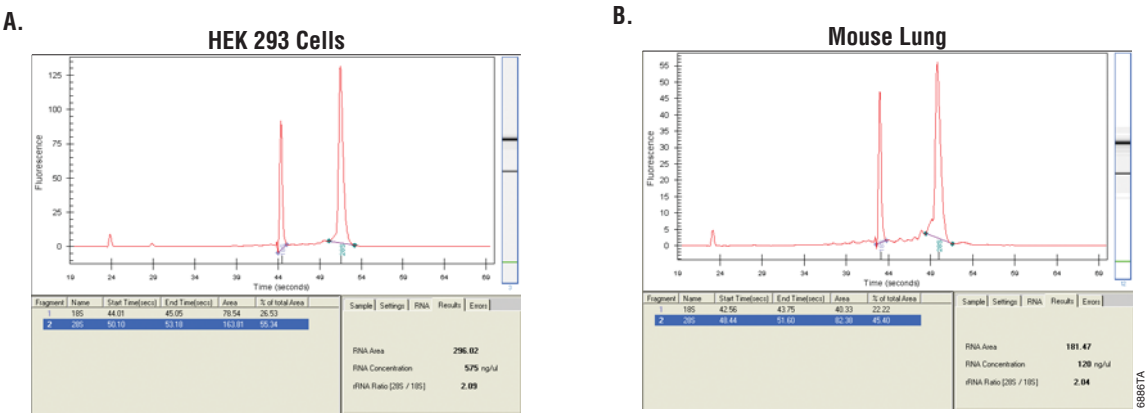

Figure 3. Agilent bioanalyzer results for total RNA purified from cells or tissue. Purified total RNA (1 µl) was assayed on the Agilent 2100 bioanalyzer with an RNA 6000 Nano LabChip®. **Panel A.** Total RNA from 1 × 10^6 HEK 293 cells (RIN = 10.0). **Panel B.** Total RNA from 10 mg mouse lung tissue (RIN = 9.8).

The Maxwell® 16 LEV Systems combine compact instrumentation, optimized automated methods, prefilled reagent cartridges, service and support to save time, increase productivity and improve the consistency of results.

CONCLUSIONS

The Maxwell® 16 System combines compact instrumentation, optimized automated methods, prefilled reagent cartridges, service and support to save time, increase productivity and improve the consistency of results. The compact design and simple operation of the Maxwell® 16 Instrument provides maximal performance and ease-of-use for low- to moderate-throughput purification of biomolecules. The Maxwell® 16 Cell and Tissue LEV Total RNA Purification Kits consistently provide high-concentration total RNA from up to 25 mg of mammalian tissue or 1 × 10^6 eukaryotic cultured cells with <10 copies gDNA/100 ng RNA (tissue) and <50 copies gDNA/100 ng RNA (cells). We have demonstrated the removal of contaminating gDNA and lack of cross-contamination by qPCR. The Maxwell® 16 Integrated System is simple to use and provides superior performance for the low- to moderate-throughput user.

REFERENCES

1. Kephart, D. *et al.* (2006) *Promega Notes* **92**, 20–3.
2. Imbeaud, S. *et al.* (2005) *Nucleic Acids Res.* **33**, e56.

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**

ADDITIONAL RESOURCES

For additional details visit:
www.promega.com/PN97Maxwell16

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

Available Separately

Product	Size	Cat.#
Maxwell® 16 Instrument*	1 each	AS2000
Maxwell® 16 LEV Instrument*	1 each	AS1002
LEV Conversion Kit for Maxwell® 16	1 each	AS1250

* For Laboratory Use.

(a)U.S. Pat. Nos. 6,027,945, 6,368,800, and 6,673,631 Australian Pat. No. 732756 and other patents and patents pending.