High-Performance RNA Isolation Using the Maxwell™ 16 Total RNA Purification Kit

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 the Maxwell™ 16 Total RNA Purification Kit to provide highly pure total RNA from a variety of sample sources including mammalian tissue, eukaryotic tissue culture cells, white blood cells, plant leaf material, or RNA-cleanup applications. The Maxwell™ 16 Instrument will process up to 16 samples in about 30 minutes of hands-free instrument operation. Purified RNA is ready for immediate downstream analysis without the need for precipitation, DNase treatment, or other post-purification manipulation. In this article we demonstrate the use of the Maxwell™ 16 integrated system for total RNA purification.

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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 quantitative RT-PCR[a] (qRT-PCR) or microarray analysis to monitor dynamic changes in gene expression during cellular growth in response to metabolic demands or environmental challenges. While the isolation of high-quality RNA provides the foundation for subsequent generation of meaningful experimental information, the actual process of isolating RNA can be tedious, complex and labor-intensive. High-throughput screeners often turn to automation to increase their throughput and minimize the labor associated with RNA purification. The Maxwell™ 16 System was developed to meet the needs of low- to moderate-throughput users by providing automated purification at a scale more appropriate to their workload without considerable capital investment, training or maintenance (1). In addition, the Maxwell™ 16 System can also be attractive for use in quarantined areas of the laboratory or for high-throughput laboratories that desire a low- to moderate-throughput integrated automated system to complement their existing instrumentation.

Ready to Use
The Maxwell™ 16 integrated system combines compact instrumentation, optimized methods and prefilled cartridges to provide consistent RNA purification.

The Maxwell™ 16 Total RNA Purification Kit[b] uses prefilled reagent cartridges to provide high yields of RNA while effectively removing contaminating genomic (gDNA) from the RNA preparation. In addition to the prefilled Maxwell™ 16 RNA purification reagent cartridges, the Maxwell™ 16 Instrument automated methods allow simple operation of the instrument for either gDNA or total RNA purification using the respective prefilled reagent cartridges. The updated Maxwell™ 16 Instrument automated methods are available to current Maxwell™ 16 System users free of charge to allow adaptation of the Maxwell™ 16 System to changing needs. In this article, we will highlight the features of the Maxwell™ 16 System and demonstrate its use in the isolation and analysis of total RNA.

MAXWELL™ 16 RNA ISOLATION PRINCIPLE

The Maxwell™ 16 Instrument has a compact design to minimize the amount of laboratory space that it occupies (Figure 1). Optimized methods are preloaded on the instrument. From arrival in the lab, the Maxwell™ 16 Instrument can be unpacked and ready to purify samples in about 20 minutes.

Successful purification of intact total RNA requires the rapid disruption of the sample, inactivation of

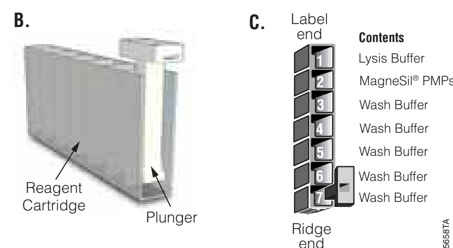

Figure 1. The Maxwell™ 16 System. The Maxwell™ 16 Instrument (**Panel A**) uses optimized reagents predispensed into disposable cartridges (**Panels B and C**) for maximized performance and convenience.

endogenous ribonucleases, removal of contaminating DNA, and purification of intact RNA. In the Maxwell™ 16 Total RNA Purification Kit protocol, samples are pre-processed by the user in about 15 minutes to create a cleared lysate. The first step involves using an RNA Lysis Buffer that contains guanidine thiocyanate (GTC) to lyse the sample (2). GTC is a chaotropic salt that disrupts nucleoprotein complexes and effectively inhibits ribonuclease activity to preserve the integrity of RNA in the sample (3). RNA Dilution Buffer is then added to samples to precipitate sample debris, and a novel Clearing Agent is added to remove contaminating gDNA. After briefly heating to 65°C, the sample is cleared by centrifugation through provided spin columns. The cleared lysate is then added directly to well #1 of the prefilled reagent cartridge. A similar protocol is followed for RNA cleanup from the aqueous phase of organic extraction-based total RNA purification methods and in vitro transcription reactions (data not shown).

The functionality of the Maxwell™ 16 Total RNA Purification Kit is based on the sequential capture and release of paramagnetic particles into the wells of the total RNA purification cartridge. RNA is efficiently captured using MagneSil® Paramagnetic Particles (PMPs), and sequential washes in alcohol-containing wash buffers effectively remove impurities. Purified total RNA is eluted from the PMPs into Nuclease-Free Water at a concentration immediately ready for many downstream applications without the need for precipitation and lengthy RNA rehydration.

SUPERIOR PERFORMANCE

The Maxwell™ 16 Total RNA Purification Kit provides an effective system for isolating total RNA. The system effectively isolates RNA from a variety of samples containing a wide range of total RNA content (Table 1). Up to 50mg of most tissue types can be prepared for purification by disruption in RNA Lysis Buffer using mechanical shearing. We have also used the system for purification of total RNA from stabilized blood, the white blood cell fraction of whole blood, and plant leaf tissue (Table 1). Protocols for optimizing RNA purification from a variety of other sample types are provided in the Maxwell™ 16 Total RNA Purification Kit Technical Bulletin #TB351 (2).

RNA yields from the Maxwell™ 16 Total RNA Purification Kit can be as much as 2- to 4-fold higher than comparable systems. For example, the measured yield for mouse liver by absorbance at 260nm is over 200µg of RNA per 50mg of tissue. All samples demonstrated high purity as measured by the A_{260}/A_{280} ratio, generally yielding ratios over 2.0. To assess the quality of the RNA listed in Table 1, we resolved aliquots of each sample by agarose gel electrophoresis under denaturing conditions and visualized each sample by ethidium bromide staining (Figure 2). Agarose gel visualization is a more accurate method of determining RNA integrity than microfluidics-based methods. The isolated RNA is of high quality and intact, as demonstrated by the sharp ribosomal bands apparent in each lane.

Tissue or Cells
The Maxwell™ 16 Total RNA Kit is effective at isolating RNA from samples with a wide range of total RNA content.

Table 1. Typical Yields and Purity of Total RNA Isolated from Tissues and Cells Using the Maxwell™ 16 Total RNA Cartridge.

Sample	Amount Processed	Typical Yield (µg)	Typical A_{260}/A_{280}
Mouse Liver	25mg	100	2.12
Mouse Liver	50mg	233	2.12
Mouse Brain	50mg	29	2.08
Mouse Heart	50mg	23	2.11
Mouse Intestine	50mg	128	2.09
Mouse Kidney	50mg	83	2.09
Mouse Lung	50mg	22	2.12
PAXgene® Tube Collected Blood Sample	1 tube	5	1.8
HeLa Cells	5×10^6	47	2.13
Tomato Leaf	100mg	33	2.19

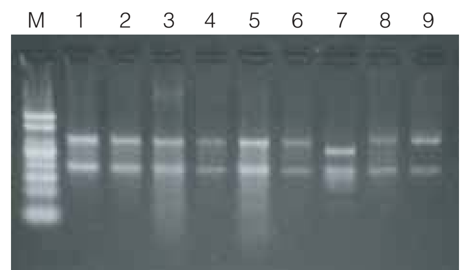

Figure 2. Agarose gel analysis of total RNA isolated using the Maxwell™ 16 Total RNA Cartridge. Total RNA was isolated from the tissues listed in Table 1. An aliquot of each sample was resolved by 1.2% agarose gel electrophoresis under denaturing conditions and visualized by ethidium bromide staining. Lane 1, mouse brain; lane 2, mouse heart; lane 3, mouse intestine; lane 4, mouse kidney; lane 5, mouse liver; lane 6, mouse lung; lane 7, tomato leaf; lane 8, PAXgene® tube stabilized human blood; lane 9, HeLa cells. Lane M, RNA Markers (Cat.# G3191).

CONSISTENT RESULTS

To demonstrate consistent performance using the Maxwell™ 16 Total RNA Purification kit, 25mg aliquots of mouse liver lysate were used for purification. The resulting RNA samples were analyzed by taking A_{260} measurements, and the yields were plotted (Figure 3, Panel A). Yields were highly consistent, with an average of approximately 100µg of RNA isolated per sample. Representative samples were also analyzed by agarose gel electrophoresis to demonstrate reproducible yield of the isolated RNA (data not shown). To demonstrate that RNA recovery is linear over a broad range of input sample mass, RNA was purified from increasing amounts of triplicate samples of mouse liver lysate. An analysis of RNA recovery (measured by absorbance at 260nm) as a function of increasing tissue mass clearly demonstrates a linear relationship over the tissue mass range examined (Figure 3, Panel B).

DNA-FREE RNA

The presence of contaminating DNA in RNA samples can lead to complications during downstream analysis. Significant DNA contamination can give rise to inaccurate quantitation of the RNA sample by spectrophotometric analysis. More importantly, highly sensitive amplification-based analysis methods such as qRT-PCR can be complicated by minute levels of genomic contamination leading to variability and doubt in the quality of your application results.

The Maxwell™ 16 Total RNA Purification Kit uses a novel method to remove gDNA contamination prior to purification of total RNA. To demonstrate the effectiveness of this technique, we used both endpoint PCR and the Plexor™ qPCR System to detect gDNA in RNA samples (Figure 4). The Plexor™ qPCR assay incorporated amplification primers designed to specifically quantitate mouse GAPDH-intron sequences. Plexor™ qPCR analysis of these 24 experimental mouse liver RNA samples demonstrated that only four of the samples contained any trace of detectable genomic DNA. These four reactions contained between 0.6 and 0.9 copies of mouse genomic DNA, resulting in an average (n=24) of less than 0.1 genome equivalents per 100ng of Maxwell™ 16-purified RNA. When analyzing representative samples of mouse liver RNA, no detectable gDNA was observed by endpoint PCR after 40 cycles of amplification. This is 100- to 1,000-fold lower gDNA contamination than DNase removal-based methods.

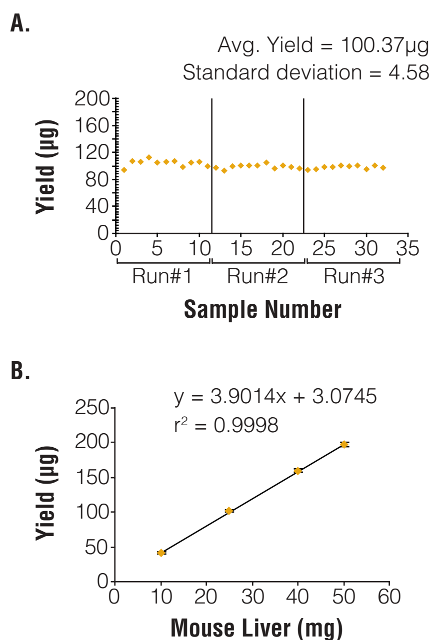

Figure 3. Consistent yield and quality of isolated total RNA. Panel A. Reproducible yields are obtained from total RNA purification using the Maxwell™ 16 Total RNA cartridge. Total RNA was isolated in three separate purification runs of at least 10 replicates from twenty-five milligrams of mouse liver lysate. Total RNA yield from each purified sample was determined by absorbance at 260nm, and the resulting values plotted. Average yield was 100.37µg with a standard deviation of 4.58µg. **Panel B.** Linear RNA recovery. Total RNA was isolated from 10, 25, 40 or 50mg of mouse liver lysate (n = 3 for each amount). Total RNA yield was determined by absorbance at 260nm and plotted against the amount of mouse liver tissue used in the purifications.

No Genomic DNA

The Maxwell™ 16 Total RNA Purification Kit results in 100- to 1,000-fold lower gDNA contamination than DNase removal-based methods.

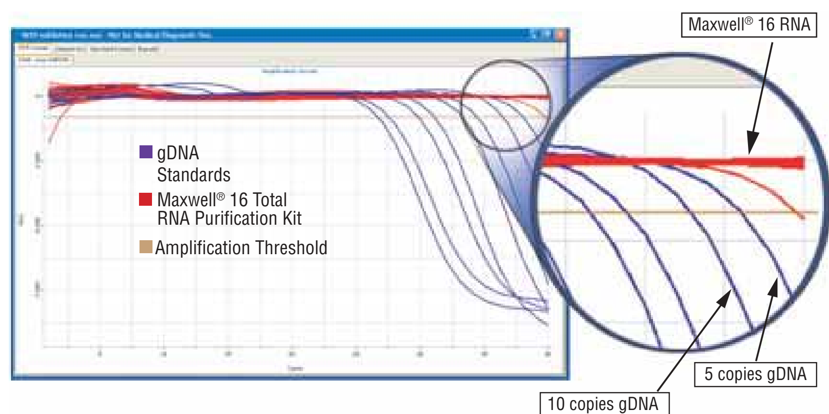

Figure 4. No detectable genomic DNA contamination. RNA was isolated from 24 separate samples of 25mg of mouse liver lysate and analyzed by endpoint and qRT-PCR. The amplification curve demonstrates the GAPDH-intron-specific Plexor™ quantitation of 10, 50, 100, 500, 1,000 and 10,000 copies of mouse gDNA standard reactions and any contaminating gDNA contained in 100ng of RNA isolated from 24 individual preparations from 25mg of mouse liver. Of the 24 individual RNA isolations, the average contaminating gDNA content was demonstrated to be 0.1 genome equivalent per 100ng of Maxwell™ 16 purified RNA.

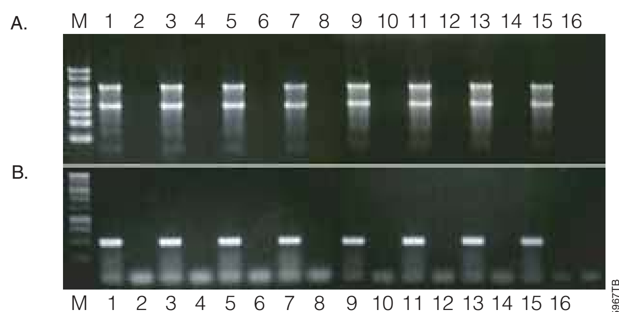

Figure 5. No detectable cross-contamination. Sixteen purification reactions were performed using an input of 25mg of mouse liver lysate (odd lanes) or SV RNA Lysis Buffer alone (even lanes). **Panel A.** Four microliter aliquots of each purified sample were resolved by 1.2% agarose gel electrophoresis under denaturing conditions. Lane M, RNA Markers (Cat.# G3191). **Panel B.** Equivalent volumes (1µl) of each sample were amplified by endpoint RT-PCR using a primer pair specific for a portion of the beta actin RNA. A total of five microliters of each amplification reaction was analyzed by 1.2% agarose gel electrophoresis and visualized by ethidium bromide staining. Varying amounts of primer-dimer are observed in each reaction. Lane M, 1kb DNA Ladder (Cat.# G5711).

Optimized
The prefilled reagent cartridges and single-use format provide strong barriers to cross-contamination during sample processing.

OPTIMIZED AUTOMATION

The prefilled reagent cartridges, single-use format and optimized automated method of the Maxwell™ 16 System provide strong barriers to cross-contamination during sample processing. To demonstrate no detectable cross-contamination, we purified 16 samples alternating input with either 25mg mouse liver lysate or RNA Lysis Buffer alone. After purification, 4µl of each sample was resolved by agarose gel under denaturing conditions (Figure 5, Panel A). Equivalent aliquots (1µl) of each sample were analyzed by endpoint RT-PCR using a primer pair specific for a region of beta actin RNA. After 35 cycles of amplification, 5µl of each sample was analyzed by gel electrophoresis (Figure 5, Panel B). No detectable cross-contamination was observed between samples.

CONCLUSIONS

The Maxwell™ 16 system combines compact instrumentation, optimized automated methods, prefilled reagent cartridges, and 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 Total RNA Purification Kit consistently provides high yields of DNA-free total RNA from up to 50mg of mammalian tissue, 5×10^6 eukaryotic tissue culture cells or 100mg plant tissue. The system can also be used to purify total RNA from a white blood cell fraction from whole blood or from stabilized solution (e.g., PAXgene® tubes). We have demonstrated the removal of gDNA to a level of less than 0.1 copy per 100ng of RNA by qRT-PCR. The Maxwell™ 16 automated integrated system is simple and provides superior performance for the low- to moderate-throughput user.

REFERENCES

1. Kephart, D. *et al.* (2006) *Promega Notes* **92**, 20–23.
2. *Maxwell™ 16 Total RNA Purification Kit Technical Bulletin* #TB351, Promega Corporation.
3. Chirgwin, J. *et al.* (1979) *BioChem.* **18**, 5294–9.

PROTOCOL

- *Maxwell™ 16 Total RNA Purification Kit Technical Bulletin* #TB351, Promega Corporation. (www.promega.com/tbs/tb351/tb351.html)

ORDERING INFORMATION

Product	Size	Cat.#
Maxwell™ 16 Total RNA Purification Kit	48 preps	AS1050
Maxwell™ 16 Instrument	1 each	AS1000

[a] Patents for the foundational PCR process, European Pat. Nos. 201,184 and 200,362, expired on March 28, 2006. In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.

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

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