

# Mini-RNA Isolation on a Large-Scale

## MagneSil® Total RNA mini-Isolation System

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### Abstract

The MagneSil® Total RNA mini-Isolation System provides a high-throughput 96- or 384-well format for fast, simple preparation of intact, purified total RNA from small amounts of cultured cells, tissue, or fresh whole blood samples. Total RNA isolation is achieved without the need for vacuum filtration, centrifugation or precipitation.

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### Introduction

We have developed a new total RNA isolation system that enables high-throughput total RNA isolation without the need for centrifugation, vacuum filtration or precipitation. The MagneSil® Total RNA mini-Isolation System<sup>(a)</sup> chemistry and format is designed for high-throughput total RNA isolation in 96-well or 384-well formats. This system takes advantage of the unique properties of the MagneSil® Paramagnetic Particle (PMP) technology<sup>(b)</sup> to isolate total RNA from complex biological mixtures.

The automated total RNA isolation procedure takes as little as 30 minutes and includes a DNase step for removal of contaminating genomic DNA. Total RNA can be isolated in 96-well plates from  $\leq 1 \times 10^5$  cell culture cells,  $\leq 2\text{mg}$  of tissue lysate, or  $\leq 20\mu\text{l}$  of whole human blood per well. Total RNA can be isolated in 384-well plates from  $\leq 1 \times 10^3$  cell culture cells or  $\leq 5\mu\text{l}$  of whole human blood per well. Purified total RNA is suitable for downstream expression profiling amplification reactions such as endpoint and realtime RT-PCR. Average total RNA yield of the MagneSil® Total RNA mini-Isolation procedure is comparable to automated vacuum filtration procedures but provides the advantages of scalability to 384-well format and flexibility of elution volume to maintain purified total RNA sample concentration for downstream applications.

### Procedure

The MagneSil® Total RNA mini-Isolation System uses the MagneSil® PMP technology. After sample lysis, nucleic acids are captured by MagneSil® PMPs. The PMPs are then washed and incubated with DNase to remove contaminating genomic DNA. The DNase is inactivated, the degraded genomic DNA is washed away, and the purified total RNA is eluted in Nuclease-Free Water. Sufficient reagents are supplied with the system to isolate total RNA from four 96-well or 384-well plates. All components are guaranteed free of contaminating ribonucleases when used as directed and are thoroughly tested to ensure optimal performance. A summary of the procedure is provided in Figure 1.

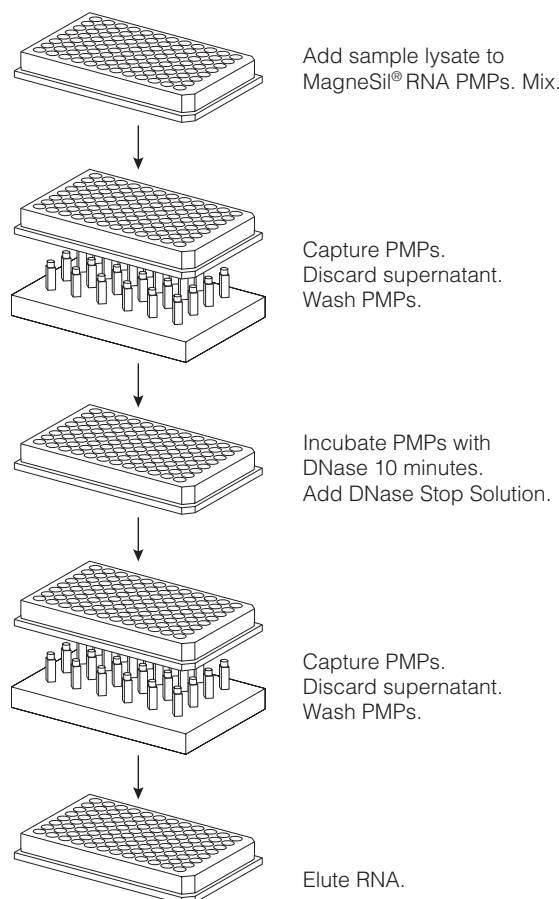
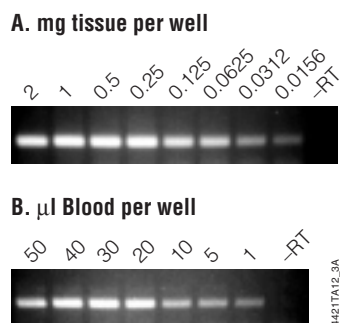


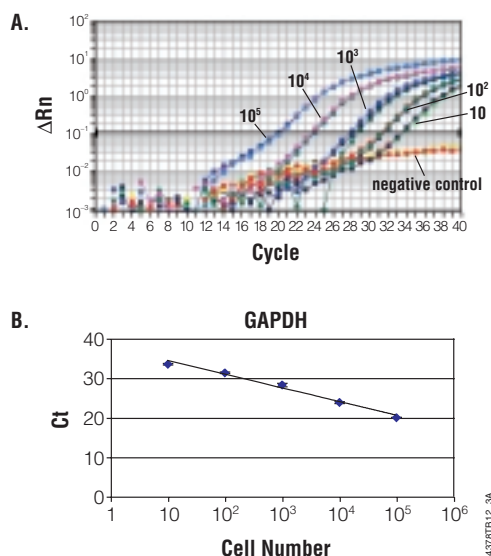
Figure 1. Schematic diagram of the MagneSil® Total RNA mini-Isolation System protocol.

## Purification of Total RNA From Cell Culture, Tissue Lysate, and Whole Blood Samples

The MagneSil® Total RNA mini-Isolation System is designed for the isolation of total RNA from small samples of cultured cells, tissue lysates, or whole blood samples. Isolation of total RNA from tissue lysates and whole blood is demonstrated by endpoint RT-PCR analysis of purified total RNA (Figure 2). Isolation of total RNA from cultured cells is demonstrated by real-time RT-PCR analysis of purified total RNA (Figure 3). Table 1 shows input sample limitations for purification of total RNA from these sample sources in 96- or 384-well formats.



**Figure 2. Endpoint RT-PCR amplification.** RNA was isolated from titration of mouse liver lysate (**Panel A**) or whole human blood (**Panel B**). One microliter of isolated RNA from each sample was analyzed by RT-PCR amplification using beta-actin primer pair. Total RNA yield is dependent upon the white cell concentration of the whole blood sample.



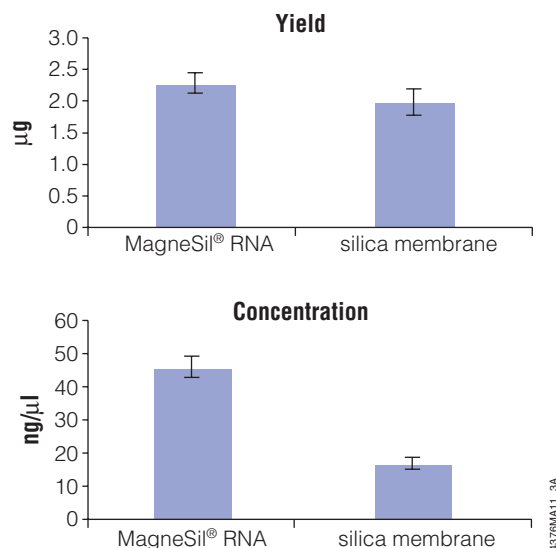
**Figure 3. Realtime RT-PCR analysis of purified total RNA.** Reverse transcription reactions (100µl) used 20µl aliquots (n = 3) of total RNA isolated from a 10X dilution series of HeLa cells ( $1 \times 10^5$  to 10 cells) seeded per well in a 96-well plate as a template. Five microliter aliquots of the RT reaction were used for PCR of a GAPDH target. **Panel A.** Sensitivity illustrated by GAPDH transcript detection in as few as 10 HeLa cells seeded/well. **Panel B.** Linear regression line fit to the data shows linear purification of total RNA over cell titration. The threshold cycle (Ct) is the cycle in which the signal rises above background.

**Table 1. Maximum Amount of Starting Material for the MagneSil® Total RNA mini-Isolation System.**

Sample Type	96-Well Format	384-Well Format
Cultured cells	$1 \times 10^5$	$1 \times 10^3$
Tissue lysate (mg tissue in 100µl lysis buffer)	2mg	Not recommended
Whole blood sample	20µl	5µl

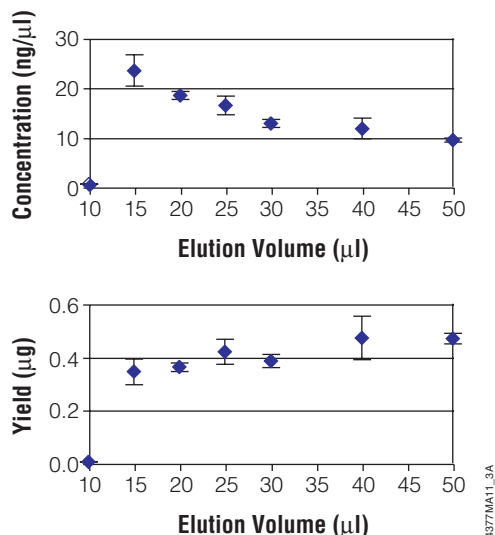
## Purification of Higher Concentration of RNA Compared to Vacuum-Format Systems

MagneSil® PMPs provide a solid, mobile phase for binding total RNA compared to the solid, fixed membrane of vacuum systems. The mobile binding surface of the MagneSil® PMPs provides equivalent performance in terms of yield as compared to vacuum-format systems while enabling elution of more concentrated purified total RNA (Figure 4). However, the MagneSil® PMPs allow elution in smaller, scalable volumes without having to sacrifice yield (Figure 5).



**Figure 4. Total RNA isolated from  $1 \times 10^5$  HeLa cells per well using the MagneSil® Total RNA mini-Isolation System or a 96-well vacuum-format total RNA isolation system.** Total RNA concentration and yield calculated by measurement of isolated total RNA using Molecular Probes RiboGreen® assay.

# MagneSil® Total RNA mini-Isolation System...continued



**Figure 5. Flexibility in elution volume to control sample concentration.** Total RNA isolated from  $2 \times 10^4$  HeLa cells/well using MagneSil® Total RNA mini-Isolation System in a 96-well format. Titration of elution volume shown. Total RNA concentration and yield calculated by measurement of isolated total RNA with Molecular Probes RiboGreen® assay.

## Sensitivity and Quality of Purified Total RNA

Total RNA purified with the MagneSil® Total RNA mini-Isolation System was evaluated by realtime RT-PCR analysis. The System is linear over a range of 10 to  $10^5$  cells used as starting material (Figure 3, Panel A). We isolated detectable amounts of RNA from as few as 10 cells (Figure 3, Panel B).

## MagneSil® Total RNA mini-Isolation System Enables Scalable-Throughput Total RNA Isolation

The MagneSil® Total RNA mini-Isolation System protocol enables manual purification with the use of a multichannel pipettor and plate shaker or high-throughput automated purification on a variety of liquid-handling workstations to meet a variety of throughput needs. Validated automated methods for the Beckman Coulter Biomek® 2000 and FX for 96- and 384-well automated total RNA isolation are available from Promega at: [www.promega.com/automethods/](http://www.promega.com/automethods/)

## Conclusions

The MagneSil® Total RNA mini-Isolation System provides conveniently formatted reagents for total RNA isolation from small sample sizes. The MagneSil® Total RNA mini-Isolation System is ideal for purification from small sample sources because of the ability to scale the elution volume without sacrificing yield. The RNA purification with the MagneSil® Total RNA mini-Isolation System is sensitive and linear. The purified RNA is suitable for downstream amplification applications such as realtime RT-PCR.

## Protocols

- ◆ *MagneSil® Total RNA mini-Isolation System Technical Bulletin #TB328*, Promega Corporation. ([www.promega.com/tbs/tb328/tb328.html](http://www.promega.com/tbs/tb328/tb328.html))



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## Ordering Information

Product	Size	Cat.#
MagneSil® Total RNA mini-Isolation System <sup>(a)*</sup>	4 plates	Z3351
MagnaBot® 96 Magnetic Separation Device	1 each	V8151
1/4 inch Foam Spacer	1 each	Z3301
Collection Plates	4 plates	A9161
Nuclease-Free Water*	50ml	P1193
MagnaBot® 384 Magnetic Separation Device*	1 each	V8241
384-Well Plate, Flat	10 plates	V5291

\*For Laboratory Use.

<sup>(a)</sup>U.S. Pat. No. 6,218,531, Australian Pat. No. 745185 and other patents pending.

<sup>(b)</sup>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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