High-Throughput. High-Quality. High Time!

High-Throughput Purification Using the SV 96 Total RNA Isolation System

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Abstract

This article introduces the SV 96 Total RNA Isolation System. The system is used to purify high-quality total RNA with a flexible method that can be performed manually or automated on a liquid handling workstation such as the Beckman Biomek® 2000 or Biomek® FX. The unique design of the SV 96 Total RNA Isolation System eliminates waste handling, includes a DNase treatment step to eliminate genomic DNA and allows consistent and reliable recovery of total RNA from tissue culture cells. The purified RNA is functionally tested in RT-PCR and results are shown to be consistent with those obtained using other RNA purification methods.

The SV 96 Total RNA Isolation System is a high-throughput system with a flexible format that allows either manual or automated total RNA isolation.

Introduction

As research moves from genome sequencing to analysis of how genes function, the need for a high-throughput method to rapidly isolate high-quality RNA, substantially free of genomic DNA contamination, from small amounts of starting material (i.e., cultured cells) has increased. The SV 96 Total RNA Isolation System has been designed to address this need. The system provides a fast, simple technique for the preparation of purified, intact RNA that allows automation on liquid handling workstations such as the Beckman Biomek® 2000 and Beckman Biomek® FX. Total RNA can be purified from 96 samples at once in less than an hour. The SV 96 Total RNA Isolation System also incorporates a DNase treatment step designed to substantially reduce genomic DNA contamination, which can interfere with amplification-based methodologies. Purification is achieved without phenol/chloroform extractions or ethanol precipitations and there is no DNase carryover in the final RNA preparation.

Procedure

The SV 96 Total RNA Isolation System purifies total RNA from cell lysates using 96 well vacuum filtration steps that eliminate the need for centrifugation (Figure 1). Sample lysate is applied to the binding plate and total RNA is captured. Washing of bound RNA requires no disassembly of the manifold, and filtrate waste products are delivered directly to a vacuum trap, eliminating the need to empty waste collection vessels during purification. DNase treatment occurs while the RNA is bound to the plate. After DNase incubation, DNase is inactivated by DNase Stop Solution, and the bound total RNA is washed again to remove digested genomic DNA. Total RNA is eluted from the Binding Plate using Nuclease-Free Water and collected into a 96 well plate.

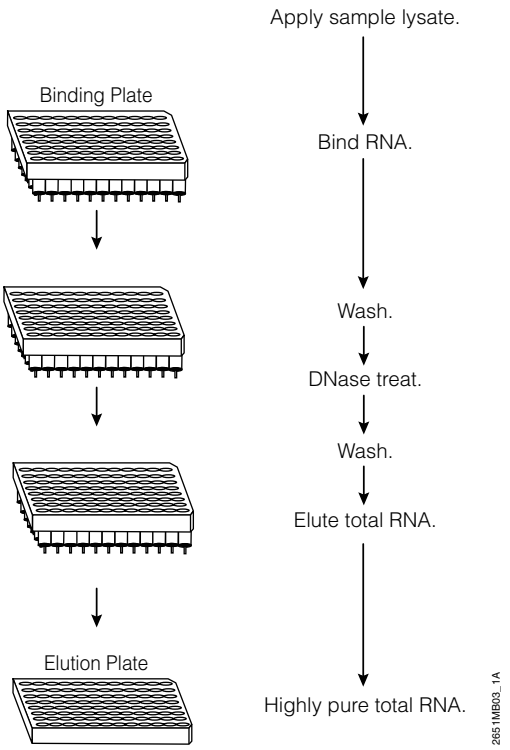

Figure 1. Total RNA isolation and purification using the SV 96 Total RNA Isolation System.

Total RNA Isolation from Tissue Culture Cells

Total RNA purified from three common cell lines was analyzed for yield and purity by measuring sample absorbance at 260 and 280nm. Total RNA yield was calculated by the absorbance at 260nm. Purity was estimated from the ratio of the absorbance at 260 and 280nm (i.e., A_{260}/A_{280}). Pure RNA exhibits an A_{260}/A_{280} ratio of 2.0. Table 1 shows the average yield and purity of total RNA purified from approximately 1×10^5 HeLa, NIH3T3 and CHO cells using the SV 96 Total RNA Isolation System.

Table 1. Average Yield and Purity of Total RNA Purified from 1×10^5 HeLa, NIH3T3 and CHO Cells.

Cell Type	Average Yield (µg)	Average A_{260}/A_{280}
HeLa	0.45 ± 0.08	2.0 ± 0.1
NIH3T3	0.36 ± 0.09	1.9 ± 0.2
CHO	0.33 ± 0.08	1.9 ± 0.2

To benchmark this new high-throughput RNA isolation system, we compared RNA yield using the SV 96 Total RNA Isolation System to that obtained using Promega's SV Total RNA Isolation System (Cat.# Z3100). Total RNA was purified from 1×10^5 HeLa, NIH3T3, or CHO cells. RNA yield and purity were not significantly different between the two preparations (data not shown).

The limit of detectable total RNA purified using the SV 96 Total RNA Isolation System was assayed by isolating total RNA from a 2X dilution series of SH-SY5Y human neuroblastoma cells. Four microliters of purified total RNA were amplified by RT-PCR using β-Actin Primer Pair[a] (Cat.# G5740) and the Access RT-PCR System[a,b] (Cat.# A1250). Amplified β-actin transcript was detectable in total RNA purified from as little as 391 cells (Figure 2).

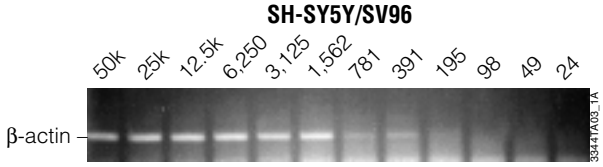

Figure 2. Limit of detection of total RNA purified from a 2X dilution series of human neuroblastoma cells. Total RNA was isolated from a human neuroblastoma cell line (SH-SY5Y cells) using the SV 96 Total RNA Isolation System. Four microliters of eluted RNA was amplified using β-Actin Primer Pairs (Cat.# G5740). PCR products were resolved on a 2% agarose gel and visualized by ethidium bromide staining. Lane headings indicate the number of cells per isolation, from 50,000 to 24. Signal is clearly present down to 391 cells.

Total RNA Isolation on the Biomek® 2000 and FX Robotic Workstations

Automation of the SV 96 Total RNA Isolation System on either the Biomek® 2000 or Biomek® FX is a "walk-away" method that can be completed in less than one hour per plate. The automated total RNA purification method is the same as the manual method described above. The initial deck configuration for the Biomek® 2000 method is shown in Figure 3. RNA yields obtained using automated formats do not differ significantly from those obtained with the manual method (data not shown). The automated isolation method reproducibly purifies intact, high-quality RNA (Figure 4, Panel A) that may be amplified by RT-PCR. Additionally, there is no detectable cross-contamination between samples purified using these automated methods (Figure 4, Panel B).

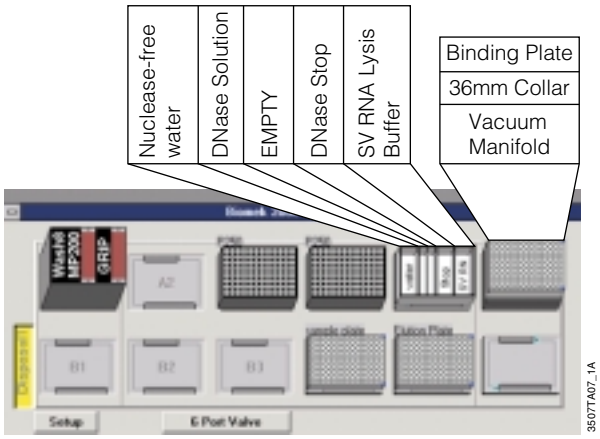

Figure 3. Deck configuration for performing RNA isolation on the Biomek® 2000 using the SV 96 Total RNA Isolation System. Tools required include Wash 8, MP200, and Gripper all placed at position A1. Positions A2, B1, B2, and B3 on the deck remain empty. Positions A3 and A4 contain boxes of P250 tips. Position A5 contains reservoirs holding Nuclease-Free Water, DNase I Solution, DNase Stop Solution, and SV RNA Lysis Buffer. The vacuum assembly is at position A6. The vacuum assembly contains a Beckman vacuum manifold with a Beckman 36mm collar stacked on the vacuum manifold and the binding plate stacked on the 36mm collar. Position B4 holds a 96 well tissue culture plate containing cells washed with PBS. Position B5 holds a 96 well elution plate. Position B6 holds a collar holder used for vacuum assembly disassembly and reassembly for total RNA elution from binding plate.

SV 96 Total RNA Isolation System Product Applications

Although the SV 96 Total RNA Isolation System was developed for high-throughput RNA isolation from tissue culture cells, it can be used for isolation of RNA from other materials. Protocols for isolation of RNA from mouse liver lysate and plant tissue lysate are given below. Plant tissue and mouse liver lysates may be stored at –70°C and may be subjected to multiple freeze-thaw cycles, however after multiple freeze-thaw cycles, total RNA yields will drop with each cycle.

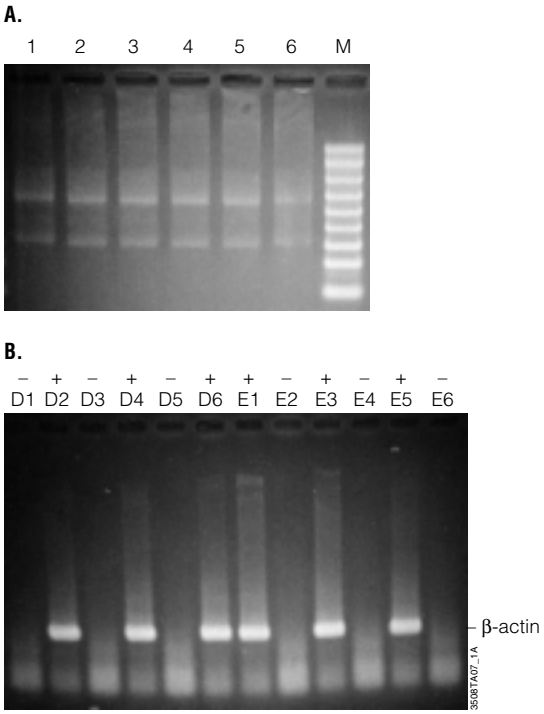

Figure 4. Automated total RNA purification on the Beckman Biomek® 2000 Robotic Workstation. Panel A: Total RNA was prepared from 1×10^6 CHO cells per well in a 96 well plate using the SV 96 Total RNA Isolation System. Twenty microliters of purified RNA from each of 6 separate isolations (lanes 1–6) were run on a 1% agarose gel and stained with ethidium bromide. Lane M, RNA Markers, 0.28–6.58kb(b) (Cat.# G3191). **Panel B:** Cross-contamination assay. Lysate prepared from 5×10^5 CHO cells (+) was arrayed in wells of a 96 well plate between wells containing an equal volume of water (–). Total RNA was prepared from all wells using the SV 96 Total RNA Isolation System. β-actin was amplified using 1µl of eluted material from each well using the Access RT-PCR System (25µl reaction volume). Ten microliters of the final RT-PCR product from wells D1–D6 and E1–E6 were run on a 1.5% agarose gel and visualized by ethidium bromide staining.

Total RNA isolation from mouse liver lysate: Sample lysate was prepared by homogenizing 30mg of mouse liver in a total volume of 1.5ml SV RNA Lysis Buffer. The lysate was kept on ice during use. The lysate (100 or 150µl) was applied directly to the binding plate and a vacuum applied until all material passed through the plate. Total RNA was then purified according to the *SV 96 Total RNA Isolation System Technical Bulletin* (#TB294). Purified total RNA was eluted with 100µl of Nuclease-Free Water. An aliquot (20µl) of the purified RNA was run on a 1% agarose gel and visualized by ethidium bromide staining (Figure 5, Panel A).

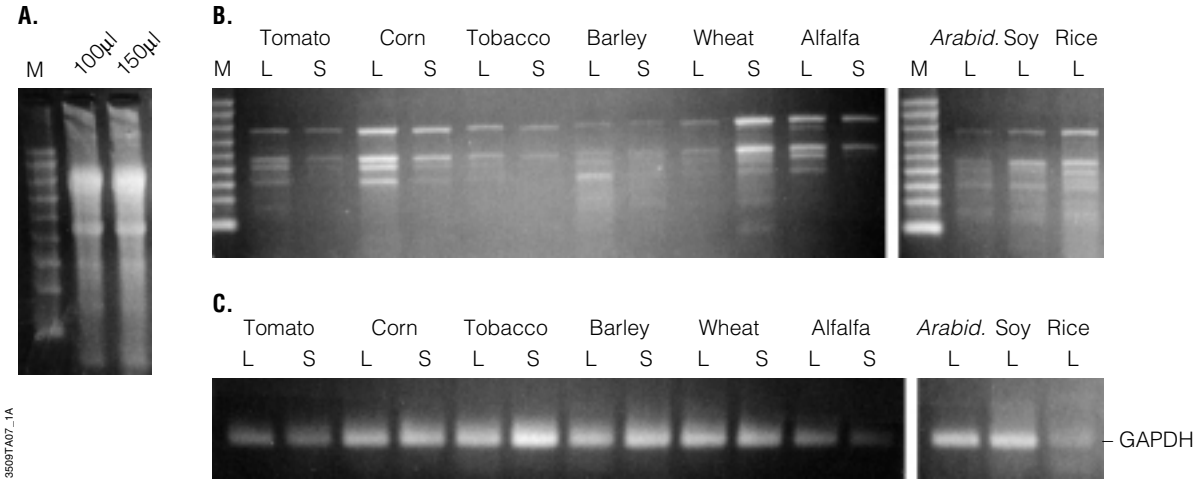

Figure 5. SV 96 Total RNA Isolation System product applications. Panel A: Isolation of RNA from mouse liver tissue. Total RNA was purified from either 100 or 150µl (2 or 3mg) of prepared mouse liver lysate. **Panel B:** Isolation of total RNA from indicated plant tissue samples (L = leaf, S = stem). Bands represent 28S and 18S rRNA, and chloroplast rRNAs. In Panels A and B, all lanes contain 20µl of isolated total RNA. Samples were separated on 1% agarose gels and visualized by ethidium bromide staining. Lanes M, RNA Markers, 0.28–6.58kb (Cat.# G3191). **Panel C:** Amplification of plant GAPDH RNA by RT-PCR from purified total RNA. One microliter of prepared total RNA was amplified using the Access RT-PCR System (Cat.# A1260) and universal plant GAPDH primers to conserved nucleotide regions of known cDNA sequences from tomato (GenBank® U97257), potato (GenBank® U17005), zea mays (GenBank® X07156) and bread wheat (GenBank® AF251217). Reactions without reverse transcriptase were performed as controls for DNA contamination. Thermal cycling conditions were: 45 minutes at 48°C, 2 minutes at 95°C, followed by 40 cycles of: 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds finishing with a 2-minute incubation at 72°C. All lanes contain 20µl of reaction product, separated on a 1.5% agarose gel. RT-PCR products were visualized by ethidium bromide staining.

Total RNA isolation from plant tissue: Sample lysate was prepared by crushing 100mg of plant tissue to a fine powder in liquid nitrogen with a mortar and pestle. SV RNA Lysis Buffer (1.5ml) was added to the tissue powder in the mortar after the liquid nitrogen evaporated. The tissue was further crushed in the SV RNA Lysis Buffer until particles and fibers were no longer visible. The lysate was then transferred to a new 1.5ml microcentrifuge tube.

Alternatively, plant tissue may be automatically ground in a 96 well format in the presence of SV Total RNA Lysis Buffer using a Retsch MM300 Mixer Mill (Retsch) or Geno/Grinder® Mill (SPEX CentriPrep, Inc.) One hundred microliters of lysate was applied directly to the binding plate and a vacuum applied until the lysate passed through the plate. Total RNA was then purified according to the SV 96 Total RNA Isolation System Technical Bulletin (#TB294). Purified total RNA was eluted with 100µl of nuclease-free water, and a 20µl aliquot was run on a 1% agarose gel and visualized by ethidium-bromide staining (Figure 5, Panel B). The purity of the total RNA was assayed by RT-PCR amplification of GAPDH (Figure 5, Panel C). Expected GAPDH RT-PCR products (~277bp) were amplified from tomato, corn (seedling), tobacco, barley, wheat, alfalfa, *Arabidopsis* leaf, mature soybean leaf and rice leaf. Control reactions without reverse transcriptase did not show amplification products, indicating no DNA contamination (data not shown).

Conclusions

The SV 96 Total RNA Isolation System is a high-throughput total RNA isolation system with a flexible format that allows either manual or automated total RNA isolation. The system provides consistent and reliable recovery of high-quality total RNA that is appropriate for amplification-based methodologies.

Protocol

- *SV 96 Total RNA Isolation System Technical Bulletin #*TB294, Promega Corporation. (www.promega.com/tbs/tb294/tb294.html)

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Applications Scientist

Ordering Information

Product	Size	Cat.#
SV 96 Total RNA Isolation System*	1 × 96	Z3500
	5 × 96	Z3505
Vac-Man® 96 Vacuum Manifold	each	A2291

**For Laboratory Use.*

Related Products

Product	Size	Cat.#
SV Total RNA Isolation System	50 preps	Z3100
	10 preps	Z3101
Wizard® SV 96 Plasmid DNA Purification System	1 × 96	A2250
	5 × 96	A2255

For Laboratory Use.

Technical Questions?
E-mail Promega Technical Services at:
techserv@promega.com