Q & A Technically Speaking

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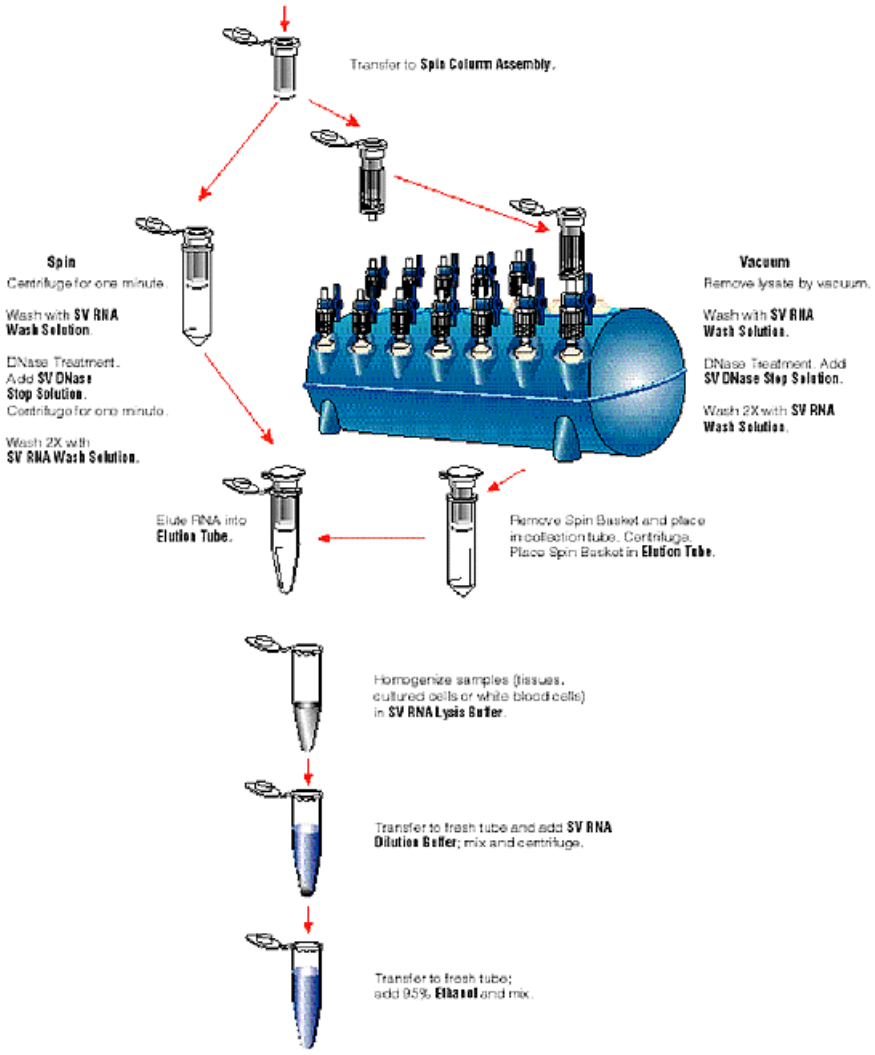
SV Total RNA Isolation System

The SV Total RNA Isolation System[a] uses Promega's unique spin/vacuum technology for isolating RNA from tissues, cultured cells or white blood cells. The isolated RNA can be used in many common downstream applications, including RT-PCR[b] and Northern blot analyses. The SV RNA System provides a fast and simple technique, in spin or vacuum format, for the preparation of purified, intact total RNA. This system also incorporates a DNase treatment step that substantially reduces genomic DNA contamination.

Q What is the SV Total RNA Isolation System?

The SV Total RNA Isolation System uses Promega's unique "SV" (spin or vacuum) technology for the rapid isolation of high yields of high quality total RNA from cells, tissues and white blood cells (1). Figure 1 schematically represents the steps in the SV Total RNA Isolation System. The isolated RNA may be used in routine molecular biology applications including RT-PCR and Northern blotting. *The Miniprep Vacuum Adapters[a] (Cat.# A1331) are required for the vacuum format of the SV Total RNA Isolation System and must be purchased separately.*



Total time: 60-70 minutes.

Figure 1. Schematic representation of the SV Total RNA Isolation System (Cat.# Z3100). The protocol is explained in detail in reference 2.

Q What are the advantages of the SV Total RNA Isolation System over traditional RNA isolation methods?

Advantages of the SV Total RNA Isolation System include:

- High yields of total RNA (Table 1).
- Rapid and convenient protocol.
- Choice of spin (centrifuge) or vacuum format.
- Compatible with tissues, cells and whole blood.
- Spin Column Assemblies and Sterile Elution Tubes provided in convenient packs.

Table 1. Properties of Total RNA Isolated from Tissues and Cells Using the SV Total RNA Isolation System.

Tissue/Cell Line*	Starting Material	Yield per Prep	Yield per mg Tissue	A_{260}/A_{230}	A_{260}/A_{280}
Liver	30mg	133µg	4.4µg	2.4	1.9
Kidney	20mg	46µg	2.3µg	2.1	1.9
Heart	60mg	16µg	0.3µg	1.8	2.1
Spleen	15mg	79µg	5.3µg	2.3	1.9
Brain	60mg	39µg	0.7µg	2.1	2.1
Lung	60mg	36µg	0.6µg	2.0	2.1
Muscle	30mg	22µg	0.7µg	1.8	2.1
RAW264.7 cells	5 x 10^6 cells	51µg	--	2.0	2.0

**The heart and lung tissues are from rat; other tissues are from mouse. The mouse macrophage cell line, RAW264.7, was grown to confluence in Dulbecco's modified Eagle's medium with 10% fetal bovine serum and 1mM pyruvate. Values in the last four columns are averages. The means for the cell line and spleen samples are the averages of two and three determinations, respectively. The means for all other samples are the averages of at least six determinations.*

Q When using the SV RNA System, what is the range of RNA yield and purity from various tissues?

The values listed in Table 1 represent averages of results achieved at Promega. Yields will vary for other tissues and the metabolic state of the animal. The purified RNA is ready for use in all routine molecular biology applications including RT-PCR and Northern blotting.

Q Will there be genomic DNA contamination in the RNA?

If the amount of recommended starting material is not exceeded, the DNase I step ensures minimal carryover of genomic DNA. Total RNA purified using this system has been shown to have undetectable amounts of genomic DNA in RT-PCR analysis with 50ng of starting RNA.

If sample amounts in excess of the recommended values listed in Table 1 are processed with the SV Total RNA Isolation System, there may be genomic DNA carryover. If traces of contaminating genomic DNA in the purified RNA are a concern, the isolated RNA can be treated with RQ1 RNase-Free DNase (Cat.# M6101) followed by phenol:chloroform extraction and ethanol precipitation.

Q Can the SV Total RNA Isolation System be used to isolate RNA from plant tissue, bacteria or yeast?

The SV Total RNA Isolation System has been used successfully to isolate RNA from leaves of tobacco, tomato and *Arabidopsis*. It can be used for isolating RNA from both Gram positive and negative bacteria. Also, a slightly modified protocol is successful for isolating

total RNA from yeast. Detailed protocols for these applications are available by contacting Promega Technical Services.

Q Can the SV Total RNA Isolation System be used to isolate genomic DNA?

The SV Total RNA Isolation System can be used to purify genomic DNA by simply omitting the DNase step in the standard protocol. Yields of 0.75-1.5µg of DNA per milliliter of blood have been obtained with this system, and the DNA can be used in amplification protocols. RNA will also be copurified.

Q Can I use elution volumes less than those recommended in the protocol?

The recommended elution volume is 50µl or greater. Elution of RNA in 25µl of water results in a decreased RNA yield (see Table 2). If more concentrated RNA samples are needed, the RNA can be dried in a Speed Vac® and resuspended in the desired volume.

Table 2. Example of RNA Yields in Various Elution Volumes.

Elution Volume (µl)	Total RNA Yield (µg)	A_{260}/A_{230}	A_{260}/A_{280}
25	101.4	2.25	2.01
50	136.9	2.33	1.95
100	137.9	2.39	1.85
200 (2 x 100)	141.0	2.43	1.75
200	131.0	2.44	1.73

Values in columns 2-4 are the average of duplicate samples.

Q How do I quantitate RNA yield and purity?

If sufficient quantities of RNA are available, the yield of total RNA obtained may be determined by spectrophotometry at 260nm (A_{260}), where 1 absorbance unit equals 40µg of single-stranded RNA/ml.

Pure RNA exhibits an A_{260}/A_{280} absorbance ratio of 2.0. The A_{260}/A_{230} ratio will also provide information on the purity of the sample. An A_{260}/A_{230} ratio less than 2.0 indicates that guanidine thiocyanate or beta-Mercaptoethanol from the Lysis Buffer is still present. If this is the case, precipitate the RNA with an acetate salt and ethanol (RNase-free).

Q Promega offers the RNAgents® System and the SV RNA System for total RNA purification. How do I choose the appropriate RNA purification system?

Both RNA isolation systems are excellent for RNA needed in many downstream applications including RT-PCR, cDNA library construction and Northern blotting.

The SV Total RNA Isolation System is a quick, column-based system (spin or vacuum format) for low amounts of starting material (see Table 1). It does **not** require phenol:chloroform extractions or salt/ethanol precipitations. A DNase I step is incorporated in the isolation procedure resulting in RNA substantially free of contaminating genomic DNA.

The RNAgents® Total RNA Isolation System is a guanidine and acidic phenol-based system and can be used to isolate RNA from up to 6g of tissue (3). The RNAgents® System has two protocols--a standard protocol that takes approximately 2.5-3 hours and a shorter (90 minutes), modified protocol for RNA to be used in RT-PCR.

REFERENCES

1. Brisco, P., Sankbeil, J. and Kephart, D. (1997) *Promega Notes* **64**, 7.
2. *SV Total RNA Isolation System Technical Manual* #TM048, Promega Corporation.
3. *RNAgents® Total RNA Isolation System Technical Bulletin* #TB087,

Promega Corporation.

Ordering Information

Product	Size	Cat.#
SV Total RNA Isolation System	50 preps	Z3100
SV Total RNA Isolation System, Trial Size	10 preps	Z3101
Miniprep Vacuum Adapters	20/pack	A1331
SV RNA Red Blood Cell Lysis Solution	200ml	Z3141

Related Products

Product	Size	Cat.#
RNAgents® Total RNA Isolation System		Z5110
RQ1 RNase-Free DNase	1,000 units	M6101

[a]*Patent Pending.*

[b]*The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.*

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.