Isolation of RNA from Plant, Yeast and Bacteria

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Editor's Note: *The following article presents data on the isolation of RNA from a number of species using the SV Total RNA Isolation System and highlights the robustness of the system. A supplemental protocol for the DNase Treatment of Total RNA Samples is available online at:* www.promega.com/tbssupp/tm048supp.html. *All Promega Technical Protocols are available at the Technical Resource Center* (www.promega.com/techserv/).

INTRODUCTION

The SV Total RNA Isolation System[a] uses Promega's unique "SV" (spin or vacuum) technology for the rapid and safe isolation of high yields of total RNA directly from cells, tissue or white blood cells. This purification is achieved without the use of phenol/chloroform extractions, and the RNA is suitable for routine molecular biology applications including RT-PCR[b] and Northern blotting.

The advantages offered by the SV RNA System include high yields of total RNA, the choice of spin or vacuum format, a fast protocol with no phenol extractions and the versatility of working with blood, tissue or cells from many species.

The tissues and cells previously tested with this system are listed in Table 1. We have now developed lysate preparation protocols for RNA isolation from plant tissue, yeast and bacteria. Figure 1 shows representative RNA isolated from these three sources. These samples require unique manipulations before starting the isolation procedures, but the remainder of the procedure is identical to the system protocol (1). The protocol modifications for each of the organisms are described below.

Table 1. Properties of Total RNA Isolated from Tissues and Cells Using the SV Total RNA Isolation System (2).

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	44µg	2.20µg	2.1	1.9
Heart	60mg	16µg	0.27µg	1.8	2.1
Spleen	15mg	79µg	5.30µg	2.3	1.9
Brain	60mg	39µg	0.65µg	2.1	2.1
Lung	60mg	36µg	0.60µg	2.0	2.1
Muscle	30mg	22µg	2.20µg	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.*

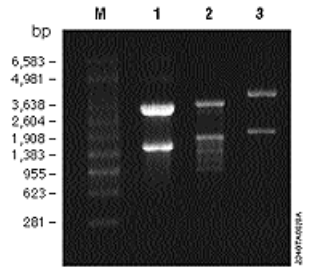

Figure 1. RNA isolated from bacteria, plant tissue and yeast. RNA samples were precipitated, resuspended in nuclease-free water and were resolved (2.5µg per sample) in a 1.5% formaldehyde/agarose gel. Lane 1, *E. coli* RNA; lane 2, tomato leaf RNA; lane 3, S. *cerevisiae* RNA. Lane M, Promega's RNA Markers, 0.28-6.58kb (Cat.# G3191).

RNA ISOLATION FROM PLANT LEAF TISSUE

1. Freeze leaf tissue in liquid nitrogen and grind using a mortar and pestle.
2. Lyse 30mg of tissue powder in 175µl of SV RNA Lysis Buffer.
3. Add 350µl of the SV RNA Dilution Buffer. Mix by inversion and microcentrifuge at maximum speed for 10 minutes.
4. Proceed with Step 2 of "RNA Purification by Centrifugation (Spin)" in the protocol provided with the system (1).

   Note: The *SV Total RNA Isolation Technical Manual* #TM048 is available online at: www.promega.com/tbs/tm048/tm048.html.

RNA ISOLATION FROM YEAST

1. Grow an overnight culture of yeast in the appropriate medium and at the appropriate temperature.
2. Make a 1:50 dilution of the overnight culture. Grow the culture to an OD_{600} value between 0.6-1.0. This normally requires 2-3 hours.
3. Centrifuge the culture at 14,000 x *g* for 2 minutes. Resuspend the pellet in 100µl of the following solution*:

1M	sorbitol
0.1M	EDTA (pH 8.0)
Add just before use:	
0.1%	beta-mercaptoethanol
50 units	Lyticase (or Zymolase)

   **Solution reagents are available from Sigma.*

4. Incubate at 30°C for 15-30 minutes. Sample should appear clear after incubation.
5. Add 75µl of SV RNA Lysis Buffer and mix gently.
6. Add 350µl of the SV RNA Dilution Buffer. Mix by inversion and microcentrifuge at maximum speed for 10 minutes at room temperature.
7. Proceed with Step 2 of "RNA Purification by Centrifugation (Spin)" in the protocol provided with the system (1).

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RNA ISOLATION FROM GRAM POSITIVE & GRAM NEGATIVE BACTERIA

The following procedure has been evaluated by using *E. coli* (Gram negative) and *B. subtilis* (Gram positive) bacteria with the centrifugation format.

1. Grow an overnight culture of bacteria in the appropriate media and at the appropriate temperature. The next day, dilute the culture 1:50 and grow to an OD_{600} of 0.6-1.0. This should take 2-3 hours. If growth is too slow, reduce the dilution factor. DO NOT use an overnight culture for direct RNA isolation. (Yields are poor if an overnight culture is used.)

2. Centrifuge 1ml of culture, in a 1.5ml tube, at 14,000 x *g* for 2 minutes at room temperature.

3. Carefully remove the supernatant, leaving the pellet as dry as possible.

4. Resuspend the pellet in 100µl of Tris-EDTA solution containing lysozyme. The final concentration of lysozyme should be 3mg/ml for Gram positive bacteria and 0.4mg/ml for Gram negative bacteria. In each case, the lysozyme solutions should be made fresh.

5. Incubate at room temperature, 5-10 minutes for Gram positive bacteria and 3-5 minutes for Gram negative bacteria.

6. Add 75µl of SV RNA Lysis Buffer to the cell extract.

7. Add 350µl of the SV RNA Dilution Buffer. Mix by inversion. DO NOT CENTRIFUGE.

8. Proceed with Step 2 of "RNA Purification by Centrifugation (Spin)" in the protocol provided with the system (1).

   Note: The *SV Total RNA Isolation Technical Manual* #TM048 is available online at: www.promega.com/tbs/tm048/tm048.html.

Table 2. Example Yields and Purity of Total RNA Isolated Using the Lysate Protocols and the SV Total RNA Isolation System.

Sample	Starting Material	Yield per Prep	Yield per mg Tissue	A_{260}/A_{230}	A_{260}/A_{280}
Plant (tomato)	30mg	4.6µg	0.15µg	1.35	2.00
Yeast (*S. cerevisiae*)	1ml OD_{600} = 0.6	19.6µg	--	1.65	2.11
Bacteria (*E. coli*)	1ml OD_{600} = 0.6	36.1µg	--	1.64	1.99

Values in columns 3-6 are averages of two samplings.

ACKNOWLEDGEMENT

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REFERENCES

1. *SV Total RNA Isolation System Technical Manual* #TM048, Promega Corporation.
2. Wiskerchen, M. (1998) *Promega Notes* **67**, 16.

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[(a)]	20 each	A1331
SV Red Blood Cell Lysis Solution	200ml	Z3141
SV RNA Lysis Buffer	50ml	Z3051

RNA Markers, 0.28-6.58kb	50µg	G3191

[(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.