

TOTAL RNA ISOLATION FROM NEURONAL CULTURES USING PROMEGA'S SV 96 BINDING PLATE ON THE BIOMEK® 2000

Randy Hoffman, B.S., Chad Zimprich, B.S.,
Georgyi Los, Ph.D., and Terri Grunst, B.S.
Promega Corporation

This article describes a protocol for the isolation of total RNA from the human neuroblastoma cell line SH-SY5Y on the Biomek® 2000 using Promega's Wizard® SV 96 Binding Plate.

Introduction

To determine if multiple neuronal samples can be processed for RNA isolation in an automated fashion, we tested the Biomek® 2000 as an automated workstation using the Wizard® SV 96 Binding Plate (Cat.# A2271) and SV Total RNA Isolation System (Cat.# Z3100) solutions.

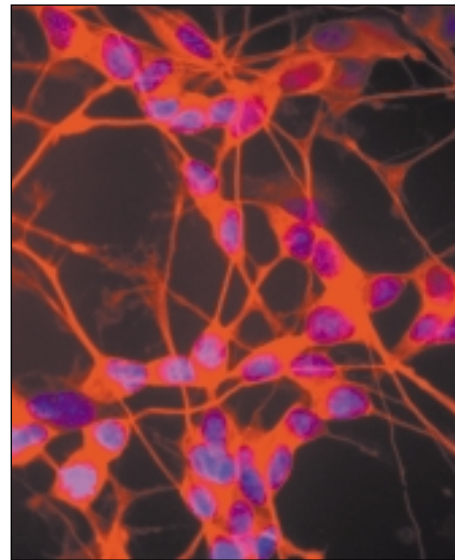
Combining the SV Total RNA Isolation System solutions with the Binding Plate on the Beckman Biomek® 2000 robotic workstation allows simultaneous processing of samples in a 96 well plate in approximately one hour. This integrated system is suitable for labs that desire processing of multiple plates in a completely automated fashion. SH-SY5Y neuroblastoma cultures were grown in serially diluted densities across a 96 well plate. After RNA isolation, RNA integrity was evaluated by RT-PCR using the β -Actin Primer Pair^(a) (Cat.# G5740).

Human SH-SY5Y cells are a pure neuroblastoma subclone of the cell line SK-N-SH derived by Dr. June Biedler (Sloan-Kettering Institute for Cancer Research, Rye, NY, USA; 1,2). These cells are widely used as an experimental model in different areas of neurobiology, including cell differentiation and cell signaling, neurotransmission and ion channel function. These cells are also used in studies addressing the molecular mechanisms of drug addiction, neurodegenerative diseases, neurotoxicity and neuropharmacology (3–8).

Promega Products Required

Product	Size	Cat. #
Wizard® SV 96 Binding Plates*	10 pack	A2271
SV RNA Lysis Buffer*	50ml	Z3051
SV Total RNA Isolation System*	50 preps	Z3100

*For Laboratory Use.



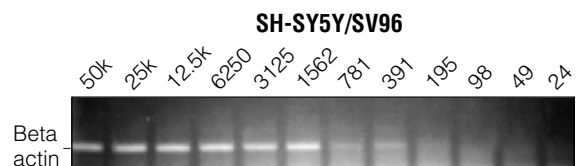
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▲ **Figure 1. Fluorescent microscopy of the human neuroblastoma cell line SH-SY5Y.** Cells were fixed with 3.7% formaldehyde, immunostained with mouse Anti- β III Tubulin mAb (Cat.# G7121) at 1.0 μ g/ml followed by an incubation with an Alexa Fluor™-594-conjugated goat-anti mouse IgG, (Molecular Probes). Cell nuclei (blue) were counterstained with DAPI (Vector Labs).



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▲ **Figure 2. Deck layout for performing RNA isolation on the Biomek® 2000 using the SV 96 Binding Plate.**



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▲ **Figure 3. β -actin RT-PCR results after RNA isolation.** Demonstration of RNA isolation from the SH-SY5Y cells using the SV 96 Binding Plate on the Biomek® 2000. Lane headings indicate the number of cells plated in that particular column from 50,000 to 24 cells. Signal is clearly present down to 391 cells.

Protocol

A. Tissue Culture

We cultured SH-SY5Y neuroblastoma cells, obtained from ATCC (CRL-2266), as described previously (4).

1. Culture cells in a 1:1 mixture of Ham's F12 nutrients and minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 100IU/ml penicillin, and 100µg/ml streptomycin in an atmosphere of 95% air and 5% CO₂ at 37°C.
2. Maintain cells at 37°C with 5% CO₂ in media (45% MEM, 45% F12, 10% FBS).
3. Determine number of viable cells using trypan blue exclusion assay.
4. Serially dilute live cells two-fold from 5 x 10⁴ cells/well to 24 cells/well, across a 96 well plate.
5. Incubate overnight at 37°C in an atmosphere of 95% air and 5% CO₂.

B. RNA Isolation

1. Remove media from the culture plate.
2. On ice, add 100µl SV RNA Lysis Buffer (Cat.# A7123) with BME to each well of the plate using a multichannel pipet. Pipet to aid in cell lysis.
3. After setting up the Biomek® 2000 deck as indicated in Figure 3, place the 96 well plate on the deck and begin the isolation program (see Table 1). Inquire with Promega Technical Services for a down-loadable Biomek® 2000 protocol for the RNA isolation procedure.
4. Store the eluted RNA on ice (immediate use), at -20°C (short-term storage) or at -70°C (long-term storage).

C. RT-PCR

1. Using the Access RT-PCR System^(a) (Cat.# A1250), set up a master mix on ice for the number of reactions (including negative control) plus one extra. For each 100µl reaction, add:

5X AMV/Tfl reaction buffer	20µl
25mM MgSO ₄	4.0µl
10mM dNTP mix	2.0µl
5' β-actin primer (5pmole/µl)	1.0µl
3' β-actin primer (5pmole/µl)	1.0µl
AMV Reverse Transcriptase (5u/µl)	2.0µl
Tfl DNA Polymerase (5u/µl)	2.0µl
Nuclease-Free Water	64.0µl

2. Add 96.0µl master mix to the PCR tube.
3. Add 4.0µl of RNA to each tube and overlay with mineral oil.
4. Run the following protocol on a thermocycler:

One cycle:	48°C for 45 minutes
One cycle:	95°C for 5 minutes
40 cycles:	95°C for 30 seconds
	60°C for 30 seconds
	70 °C for 1 minute
One cycle:	70°C for 5 minutes
One cycle:	Hold at 4°C
(Settings for a PE480 thermocycler.)	
5. Run 5µl on a 2.0% agarose gel.

Table 1. Summary of the Biomek® 2000 Program.

1. Transfer total cell lysate to the wells of the Binding Plate.
2. Apply vacuum.
3. Add 500µl SV RNA Wash Solution to each well of the Binding Plate.
4. Apply vacuum.
5. Add 25µl DNase Solution (20µl Yellow Core Buffer, 2.5µl MnCl₂, 2.5µl DNaseI) to each well of the Binding Plate.
6. Incubate DNase Solution on plate for 10 minutes at room temperature.
7. Add 200µl DNase Stop Solution to each well of the Binding Plate.
8. Apply vacuum.
9. Add 500µl SV RNA Wash Solution to each well of the Binding Plate.
10. Apply vacuum for 5 minutes to dry the Binding Plate.
11. Remove the Binding Plate from the vacuum manifold base and position on the vacuum manifold collar that is positioned over the elution plate.
12. Add 100µl Nuclease-Free Water to each well of the Binding Plate.
13. Apply vacuum. Purified total RNA is eluted in approximately 85µl Nuclease-Free Water into the 96 well elution plate.

Conclusion

We have demonstrated the ability to isolate neuronal total RNA on an automated platform, the Biomek® 2000, using the Wizard® SV 96 Binding Plate and component reagents from the SV Total RNA Isolation System. The method described here provides automated RNA isolation while addressing a particular throughput need. On the Biomek® 2000, amplifiable RNA is detected from as few as 391 SH-SY5Y cells using an RT-PCR assay. Total RNA integrity has been demonstrated in other cell models by visualization of ribosomal RNA using electrophoresis (data not shown).

References

1. Biedler, J.L. *et al.* (1978) *Cancer Res.* **38**, 3751–3757.
2. Ross, R.A., Spengler, B.A. and Biedler, J.L. (1983) *J. Natl. Cancer Inst.* **71**, 741–747.
3. Lambert, D.G. *et al.* (1992) *Prog. Neuropsychopharmacol. Biol. Psychiatry* **16**, 253–270.
4. Los, G.V., Artemenko, I.P. and Hokin, L.E. (1995) *Biochem. J.* **311**, 225–232.
5. Pahlman, S. *et al.* (1995) *Eur. J. Cancer* **31A**, 453–458.
6. Carlson K. (2000) *Toxicol. Appl. Pharmacol.* **168**, 102–113.
7. Lopez, E. and Ferrer I. (2000) *Brain Res. Mol. Brain Res.* **85**, 61–67.
8. Macleod, M.R. *et al.* (2000) *Brain Res.* **889**, 308–315.

Ordering Information

Product	Size	Cat.#
Wizard® SV 96 Binding Plate*	10 pack	A2271
	100 pack	A2278
SV Total RNA Isolation System*	50 preps	Z3100
SV RNA Lysis Buffer	50ml	Z3051
Access RT-PCR System*(a,b)	20 reactions	A1260
	100 reactions	A1250
	500 reactions	A1280
β-Actin Primer Pair ^(a)	20 reactions	G5740
Anti-βIII Tubulin mAb	100µg	G7121

*For Laboratory Use.

^(a)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. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

^(b)U.S. Pat. Nos. 4,966,964, 5,019,556, and 5,266,687 which claim vectors encoding a portion of human placental ribonuclease inhibitor are exclusively licensed to Promega Corporation.

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