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Isolating Nucleic Acids from Small Amounts of Brain Lysates Using the SV Total RNA Isolation System

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The isolation of RNA from tissue and cellular sources is a necessary first step in a diverse array of molecular biological techniques. This report investigates the use of Promega's SV Total RNA Isolation System[a] (Cat.# Z3100) with decreasing amounts of rat total brain lysates. The amount of RNA isolated was linear over the range of tissue tested. Spectrophotometric analysis and the amplification of specific neural targets demonstrate the excellent quality and performance of the isolated RNA.

INTRODUCTION

The isolation and analysis of cellular RNA is a fundamental molecular biological technique used to analyze the dynamic expression of target genes. Traditionally, the rapid isolation of high-quality total RNA devoid of contaminating genomic DNA has proved challenging due to the large amounts of ribonucleases and genomic DNA present in many target tissues. These problems are compounded in many model systems, including those of neurobiological significance, where small sample sizes also yield limited quantities of RNA.

The SV Total RNA Isolation System enables the extremely rapid and simple isolation of total RNA from many tissue and cellular sources, including total brain lysates (1). A unique feature of the SV RNA System is a simple and effective step designed to eliminate nearly all genomic DNA, a potential contaminant that can be especially troublesome when dealing with small amounts of irreplaceable RNA or a limited source of RNA. This report demonstrates the use of the SV Total RNA Isolation System to isolate RNA from decreasing amounts of rat brain lysate. Analysis of RNA from extremely small amounts of starting material can be challenging. Promega's Access RT-PCR System[b] (Cat.# A1250) was used to demonstrate the presence of specific RNA species in the equivalent of 10ng of starting tissue.

RNA ISOLATION AND ANALYSIS

Frozen rat brains were weighed and placed in SV RNA Lysis Buffer (175µl per 60mg of rat brain, frozen weight). The tissue was dispersed by manual homogenization and aliquots of the crude lysate were diluted further (in SV RNA Lysis Buffer) to the equivalent of 60, 20, 6.7, 2.2 and 0.25mg of brain per 175µl of solution. RNA was prepared from these dilute solutions using the protocol from the SV Total RNA Isolation System Technical Manual (2) and eluted in 100µl of nuclease-free water. The amount of RNA isolated from the rat brain tissue was linear over the sample sizes tested (Table 1). Quantitation of RNA by spectrophotometer reading from the 0.25mg sample was not possible due to limiting amounts of RNA isolated from the small amount of tissue.

Table 1. Purity and Yield of RNA Isolated from Brain Lysates.

Brain Lysate Processed	A_{260}	A_{280}	A_{260}/A_{280}	Yield
60mg	11.94	5.57	2.15	47.76µg
20mg	4.20	1.97	2.15	16.86µg
6.7mg	1.40	0.90	1.55	5.58µg
2.2mg	0.36	0.18	2.00	1.44µg

A unique problem when isolating nucleic acids from brain is the high lipid content of this tissue. After addition of the SV RNA Dilution Buffer and centrifugation, a lipid disc may be observed floating on top of the aqueous phase. Transfer of this lipid should be avoided as it can clog the membrane in the SV Spin Basket. To recover the aqueous phase, the lipid disc was gently pushed aside with a pipette tip. If any of the lipid is transferred, one can centrifuge the solution again and transfer the supernatant to a new tube. It is better to leave a little of the aqueous solution in the tube rather than trying to get it all and risk transferring lipids to the membrane. The use of >60mg of tissue (per 175µl lysis buffer) can also lead to clogging of the membrane. The aqueous layer is always a bit turbid during RNA isolation from brain, but a second transfer and an additional centrifugation for 5-10 minutes can be performed if lipid carryover is a concern.

Aliquots of the isolated RNA samples (4µl) were analyzed by RT-PCR using Primer Pairs[b] designed to amplify a region of rat beta-

actin, brain-derived neurotrophic factor (BDNF; Cat.# G5750) or TrkB tyrosine kinase receptor (Cat.# G5790) and Promega's Access RT-PCR System (Cat.# A1250). RT-PCR analysis was successful for all three primer pairs. Analysis by amplification is dependent on the relative abundance of the RNA being studied. The TrkB RNA target was weakly detected in the RNA isolated from 0.25mg of brain, while the BDNF and beta-actin RNA targets required 2.2mg of starting tissue for detection (Figure 1).

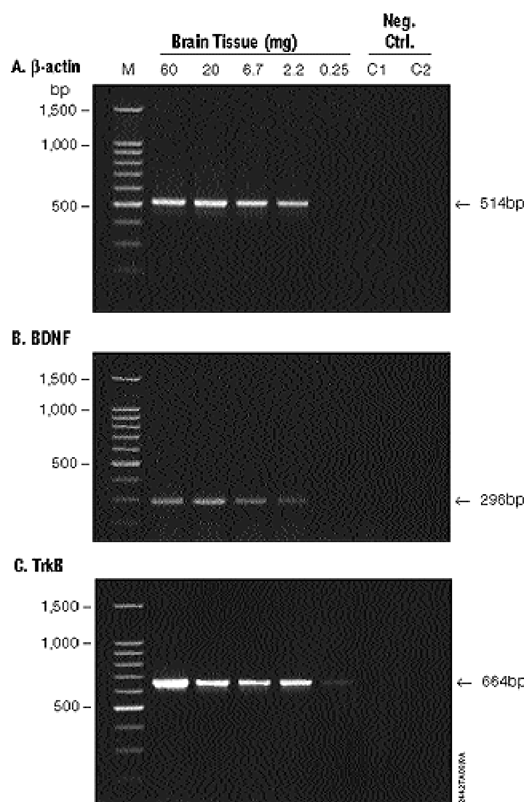

Figure 1. RT-PCR amplification of rat brain RNA. RT-PCR was performed using 4µl of each RNA preparation and Primer Pairs for beta-actin (Panel A), BDNF (Panel B) or TrkB (Panel C). The Access RT-PCR System (Cat.# A1250) was used following the supplied Technical Bulletin (3) with a final Mg^{2+} concentration of 1mM. The RNA was reverse transcribed (48°C for 45 minutes), the reverse transcriptase was inactivated (94°C for 2 minutes) and the cDNA immediately amplified using an amplification profile consisting of 40 cycles of denaturation (94°C for 30 seconds), annealing (60°C for 1 minute) and extension (68°C for 1 minute). After a final extension step (72°C for 7 minutes), the reactions were stored at 4°C. Fragments were resolved on a 2% nondenaturing agarose gel and DNA was visualized by staining with ethidium bromide. Lane M, Promega's 100bp DNA Ladder (Cat.# G2101); lane C1, no RNA; lane C2, no AMV Reverse Transcriptase.

Conclusions

This study demonstrates the utility of Promega's SV Total RNA Isolation System with limiting amounts of brain tissue. RNA yield was determined by spectrophotometry, and the RNA demonstrated excellent performance in RT-PCR. The ability to detect a particular RNA target is dependent upon the abundance of that RNA. This was demonstrated by the higher levels of RNA required for detection of the BDNF transcript compared to the ease of amplifying the abundant beta-actin and TrkB transcripts. The RNA isolation technique demonstrated here should prove useful in studies where amounts of target tissues are limited.

References

1. Brisco, P. (1997) *Promega Notes* **64**, 7.
2. *SV Total RNA Isolation System Technical Manual* #TM048, Promega Corporation.
3. *Access RT-PCR System Technical Bulletin* #TB220, Promega Corporation.

Ordering Information

Product	Size	Cat.#
SV Total RNA Isolation System	50 preps	Z3100
	10 preps	Z3101
	100 reactions	A1250

Access RT-PCR System	500 reactions	A1280
Access RT-PCR Introductory System	20 reactions	A1260
BDNF Primer Pair	20 reactions	G5750
TrkB Primer Pair	20 reactions	G5790
Beta-Actin Primer Pair	20 reactions	G5740

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

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