

# PolyAtract<sup>®</sup> Systems for mRNA Purification



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*This update summarizes the features of the PolyAtract<sup>®</sup> family of mRNA purification products. These systems employ magnetics-based separation for isolation of mRNA from cell and tissue homogenates, and from total RNA preparations. We demonstrate the utility of the PolyAtract<sup>®</sup> Systems for preparation of RNA for RT-PCR\*, and review how the PolyAtract<sup>®</sup> System 1000 can accommodate a variety of sample sizes, including samples as small as 5mg or containing as few as 10<sup>6</sup> cells.*

\*PCR is a patented process. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

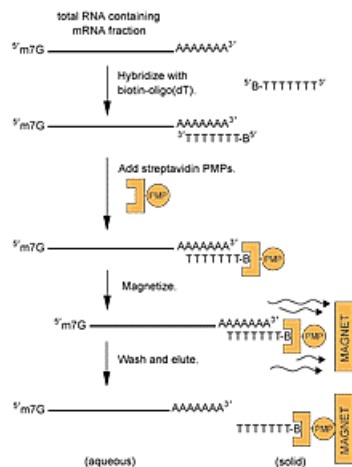
## Introduction

Isolation of mRNA from cells and tissue is complicated by the inherent instability of RNA. Traditional methods to minimize degradation and ensure maximal yields of mRNA are both cumbersome and time-consuming. Isolation of RNA normally takes several hours or more and frequently relies on the use of caustic organic solvents such as phenol (1). Furthermore, significant loss of RNA is common during the multiple precipitation and centrifugation steps required by these protocols. Oligo(dT) cellulose separation of mRNA is also time-consuming and may result in a further reduction in mRNA yield. Using magnetics-based separation technology, the PolyAtract<sup>®</sup> mRNA Isolation Systems circumvent many of these problems.

## Principles of the PolyAtract<sup>®</sup> mRNA Isolation Systems

The PolyAtract<sup>®</sup> Systems combine the affinity of biotin for streptavidin with magnetics-based separation technology (Figure 1; 2). The isolation of pure mRNA begins with the addition of biotinylated oligo(dT) probe directly to a cell or tissue lysate or to a solution of total RNA. The oligo(dT) hybridizes specifically to the 3' poly(A) region present in most mature eukaryotic mRNA species. Next, streptavidin-coated paramagnetic particles (SA-PMPs) are added which form complexes with the oligo(dT)/mRNA hybrids via the powerful affinity of biotin for streptavidin. A magnetic stand is used to capture the complexes. After washing the complexes, the purified mRNA is eluted from the solid phase by the addition of Nuclease-Free Water (Cat.# P1193). This procedure yields an essentially pure fraction of mature mRNA after only a single round of magnetic separation. The PolyAtract<sup>®</sup> Systems I-IV are designed for isolation of mRNA from a preparation of total RNA. The PolyAtract<sup>®</sup> System 1000 and PolyAtract<sup>®</sup> Series 9600<sup>™</sup> mRNA Isolation Systems\*\* allow the direct isolation of mRNA from tissue or cells (Table 1). The first step in the PolyAtract<sup>®</sup> System 1000 and PolyAtract<sup>®</sup> Series 9600<sup>™</sup> System protocols is to homogenize the cell or tissue sample(s) using GTC Extraction Buffer, which is provided with the system. This solution contains guanidine thiocyanate and beta-mercaptoethanol, powerful inhibitors of ribonuclease activity (3).

\*\*U.S. Pat. No. 5,552,302 has been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor (PRI). Inhibitors of Angiogenin, which comprises a segment of human PRI, is the subject of U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687 assigned to the President and Fellows of Harvard College and exclusively licensed to Promega Corporation.



**Figure 1. Schematic diagram of the PolyATtract<sup>®</sup> mRNA Isolation System procedure.** Cell or tissue homogenates, or total RNA samples are hybridized to oligo(dT) that is conjugated to biotin. The oligo(dT) attaches to the poly(A) tail of polyadenylated mRNA species. Streptavidin-Paramagnetic Particles (SA-PMPs), which bind the biotin moiety, are added. A magnet is used to attract the SA-PMPs, resulting in isolation of the attached complex containing mRNA. Separation of mRNA from the SA-PMP complex occurs by addition of Nuclease-Free Water, which releases the mRNA.

System	Sample Type	Sample Size	Number of Samples per System	Time to Complete the Protocol	Notes
PolyATtract <sup>®</sup> System 1000	Animal cells or tissue, plant tissues	5mg - 1g tissue; 10 <sup>6</sup> - 10 <sup>8</sup> cultured cells	2 x 1g; 350 x 5mg	30-45 minutes	System compatible with all of the MagneSphere <sup>®</sup> Technology Magnetic Separation Stands.
PolyATtract <sup>®</sup> Series 9600 <sup>™</sup> mRNA Isolation System (does not include Magnetic Separation Stand)	Animal cells or tissue	2 x 10 <sup>2</sup> - 1 x 10 <sup>6</sup> cells; 5µg - 2.5mg tissue	288 samples (3 x 96 well plates)	Z3790: ≤3 hours Z3890: ≤2.5 hours	PolyATtract <sup>®</sup> Series 9600 <sup>™</sup> Multi-Magnat <sup>®</sup> (Z3811) must be purchased separately. A centrifuge microplate carrier is required.
PolyATtract <sup>®</sup> System I	Total RNA	1-5mg	3	30 minutes	
PolyATtract <sup>®</sup> System II	Total RNA	1-5mg	3	30 minutes	Same reagents as PolyATtract <sup>®</sup> System I; includes Magnetic Separation Stand.
PolyATtract <sup>®</sup> System III	Total RNA	100-1,000µg	15	30 minutes	Same reagents as PolyATtract <sup>®</sup> System IV; includes Magnetic Separation Stand.
PolyATtract <sup>®</sup> System IV	Total RNA	100-1,000µg	15	30 minutes	

<sup>†</sup>U.S. Patent No. 5,567,326 has been issued to Promega Corporation for a multi-sample magnetic separation device.

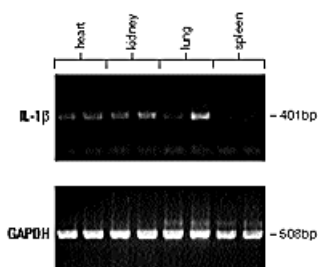
## High yields of pure mRNA

The PolyATtract<sup>®</sup> Systems offer significant benefits over conventional mRNA isolation methods. The magnetics-based protocols require minimal hands on time with completion in as little as thirty minutes. The PolyATtract<sup>®</sup> System 1000 and Series 9600<sup>™</sup> can be used to isolate mRNA directly from cells or tissue. High yields of pure mRNA are routinely obtained, as the loss of mRNA that typically occurs during the organic extractions and precipitations common to standard total RNA isolation techniques is eliminated. The use of solution-based hybridization facilitates the interaction of the oligo(dT) probes with the poly(A) tails of the eukaryotic mRNAs and also helps to ensure a maximum yield of pure mRNA, up to twice as much as that obtained using oligo(dT) cellulose-based methods. Virtually no rRNA contamination of the mRNA is observed when the PolyATtract<sup>®</sup> Systems are used. In contrast, oligo(dT) cellulose often fails to remove significant amounts of rRNA. The PolyATtract<sup>®</sup> Systems do not require the use of phenol and other organic solvents.

PolyATtract<sup>®</sup> mRNA Isolation Systems are available for purification of mRNA from tissue, cell homogenates or total RNA. Features of the PolyATtract<sup>®</sup> Systems are summarized in [Table 1](#).

## Applications of mRNA isolated using the PolyATtract<sup>®</sup> mRNA Isolation Systems

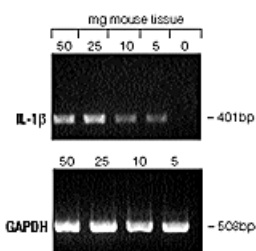
Messenger RNA purified with the PolyATtract<sup>®</sup> Systems is suitable for many molecular biology applications, including RT-PCR analysis, *in vitro* translation, cDNA synthesis and Northern blot hybridizations. We used mRNA isolated with the PolyATtract<sup>®</sup> System 1000 to investigate differential expression of IL-1beta from heart, kidney, lung and spleen of BALB/c mice. The mRNA was reverse-transcribed to cDNA and amplified using primers specific for IL-1beta, resulting in a 401bp DNA fragment. As a control, expression of the housekeeping gene GAPDH was monitored in the same tissues ([Figure 2](#)); primers for GAPDH resulted in amplification of a 508bp DNA fragment. To analyze for the presence of contaminating genomic DNA in amplification products, IL-1beta primers that span intron six were used. In the presence of genomic DNA, these primers amplify a 1,123bp DNA fragment. No band of this size was visible on any of the gels.



**Figure 2. Differential expression of IL-1beta and GAPDH in mouse tissues.** Fifty milligrams of tissue from untreated

BALB/c mice was homogenized in GTC extraction buffer and mRNA was isolated in duplicate, using the PolyAtract<sup>®</sup> System 1000. mRNA was eluted in 400µl of Nuclease-Free Water and cDNA prepared from 5% of each eluate, using conditions as described in the *PolyAtract<sup>®</sup> Series 9600<sup>™</sup> Technical Manual #TM031*. PCR was performed using primers for IL-1beta (which amplify a 401bp DNA fragment) and GAPDH (which amplify a 508bp DNA fragment). *Taq* DNA polymerase (Boehringer Mannheim) was used for amplification. PCR products were resolved on a 2% agarose gel, and visualized by staining with ethidium bromide. The primers for IL-1beta span intron 6 and amplify a 1,123bp product when genomic DNA is present in the sample. No bands at 1,123bp were seen in the cDNA amplified with IL-1beta primers.

To evaluate the ability of the PolyAtract<sup>®</sup> System 1000 to prepare mRNA from small amounts of tissue homogenates, RT-PCR was used to examine expression of IL-1beta and GAPDH message isolated from serial dilutions of mouse tissue. **Figure 3** demonstrates the expression of both genes; amplified DNA for IL-1beta and GAPDH was easily detected when mRNA was purified from as little as 5mg of mouse liver.



**Figure 3. Detection of IL-1beta and GAPDH transcripts in serial dilutions of mouse liver homogenates.** Liver tissue was harvested from untreated BALB/c mice and tissue homogenates prepared as described in **Figure 2**. The homogenates were serially diluted and the PolyAtract<sup>®</sup> System 1000 used to isolate mRNA from each dilution. cDNA was prepared from 20µl of each mRNA sample using conditions as described in the *PolyAtract<sup>®</sup> Series 9600<sup>™</sup> System Technical Manual #TM031*. PCR was performed as stated in **Figure 2**, using *Taq* DNA polymerase (Boehringer Mannheim). The resulting PCR products were separated on a 2% agarose gel and stained with ethidium bromide. Lanes correspond to the amount of liver homogenate used in the mRNA isolations.

## Size versatility of the PolyAtract<sup>®</sup> System 1000

The popularity and tremendous utility of RT-PCR brings an increasing need to process ever smaller and more numerous samples. A significant advantage of the PolyAtract<sup>®</sup> System 1000 is that it can be used for a wide variety of sample sizes; 5 milligrams to 1 gram of sample per preparation can be processed with measurable mRNA results. **Table 2 and 3** summarize the volumes of PolyAtract<sup>®</sup> System 1000 reagents required for various starting amounts of cells (**Table 2**) and tissue (**Table 3**).

Component	Cell Number	
	1 x 10 <sup>6</sup>	1 x 10 <sup>7</sup> - 1 x 10 <sup>8</sup>
GTC Extraction Buffer	200µl	4ml
Dilution Buffer	400µl	8ml
Oligo(dT) Probe	30pmol	500pmol
SA-PMP	500µl	6ml
Wash Buffer	1ml	2ml
Elution Buffer	100µl	1ml

Component	Tissue Amount (mg)*					
	5	10	25	50	100	125-1,000
GTC Extraction Buffer	40µl	80µl	200µl	400µl	800µl	4ml
Dilution Buffer	80µl	160µl	400µl	800µl	1.6ml	8ml
Oligo(dT) Probe	5pmol	10pmol	25pmol	50pmol	100pmol	1,000pmol
SA-PMP	60µl	120µl	300µl	600µl	1.2ml	12ml
Wash Buffer	500µl	500µl	1ml	1ml	1ml	2ml
Elution Buffer	40µl	80µl	200µl	400µl	800µl	6ml

\*For sample sizes that are not listed, use the next highest reagent volumes listed in this table. For example, for a 17-21mg tissue sample, use the reagent volumes listed for a 25mg tissue sample. For 125-1,000mg of tissue, adjust the oligo(dT) probe and SA-PMP volumes in proportion to the sample size, adding 5pmol of oligo(dT) probe and 60µl of SA-PMPs per 5mg increase in tissue size.

The PolyAtract<sup>®</sup> System 1000 is fully compatible with the MagneSphere<sup>®</sup> Magnetic Separation Stands (available separately), making the PolyAtract<sup>®</sup> System 1000 even more flexible; up to twelve samples may be processed at one time (**Table 4**).

Sample Size Isolated using the PolyAtract® System 1000	Tube Size	Cat. # of the MagneSphere® Magnetic Separation Stand (2-hole)	Cat. # of the MagneSphere® Magnetic Separation Stand (12-hole)
5-10mg	0.5ml	Z5331	Z5341
5-35mg	1.5ml	Z5332	Z5342
35-100mg	12 x 75mm	Z5333	Z5343
100mg - 1g	50ml*	Z5410	NA
1 x 10 <sup>6</sup> cells	1.5ml	Z5332	Z5342
1 x 10 <sup>7</sup> - 1 x 10 <sup>8</sup> cells	50ml*	Z5410	NA

NA: not applicable  
 \*Use sterile, polypropylene tubes. We recommend 15ml or 50ml centrifuge tubes with caps (e.g., Corning™ tubes) for sample sizes ≥ 3.6ml.

The PolyAtract® Series 9600™ mRNA Isolation Systems are ideal for multiple, small scale isolations of mRNA. Potential applications include the study of differential expression of genes at various stages of development. The PolyAtract® Series 9600™ System uses a 96 well plate format, which allows processing of multiple samples simultaneously, reducing potential sample-to-sample variation (4). Systems are available which include components for reverse transcription of mRNA to cDNA (see Ordering Information).

## Summary

The PolyAtract® Systems, which incorporate magnetics-based separation, offer significant advantages over conventional mRNA isolation strategies. Advantages include higher mRNA yields and reduced protocol times. The sample size versatility of these systems and their compatibility with RT-PCR further enhance their utility for molecular biology protocols requiring intact, high quality mRNA.

## References

1. Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156.
2. *PolyAtract® mRNA Isolation Systems Technical Manual #TM021*, Promega Corporation.
3. Chirgwin, J.M. *et al.* (1979) *Biochemistry* **18**, 5294.
4. Brisco, P. *et al.* (1995) *Promega Notes* **53**, 8.

## Ordering Information

Product	Cat.#	
PolyAtract® System 1000 (with Magnetic Stand)	Z5420	
PolyAtract® System 1000 (without Magnetic Stand)	Z5400	
PolyAtract® Magnetic Separation Stand	Z5410	
PolyAtract® System I (without Magnetic Stand)	Z5210	
PolyAtract® System II (with Magnetic Stand)	Z5200	
PolyAtract® System III (with Magnetic Stand)	Z5300	
PolyAtract® System IV (without Magnetic Stand)	Z5310	
PolyAtract® Series 9600™ mRNA Isolation System with cDNA Synthesis reagents	Z3790	
PolyAtract® Series 9600™ mRNA Isolation System without cDNA Synthesis reagents	Z3890	
PolyAtract® Series 9600™ Multi-Magnet	Z3811	
Product	Size	Cat.#
MagneSphere® Technology Magnetic Separation Stand (two-hole)	0.5ml	Z5331
	1.5ml	Z5332

	12 x 75mm	Z5333
MagneSphere <sup>®</sup> Technology Magnetic Separation Stand (twelve-hole)	0.5ml	Z5341
	1.5ml	Z5342
	12 x 75mm	Z5343
MagneSphere <sup>®</sup> Technology Magnetic Separation Stand, 24 well		Z5441
MagneSphere <sup>®</sup> Technology Magnetic Separation Stand, 96 well*		Z5431

\*Not compatible with the PolyATtract<sup>®</sup> Series 9600 System.

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