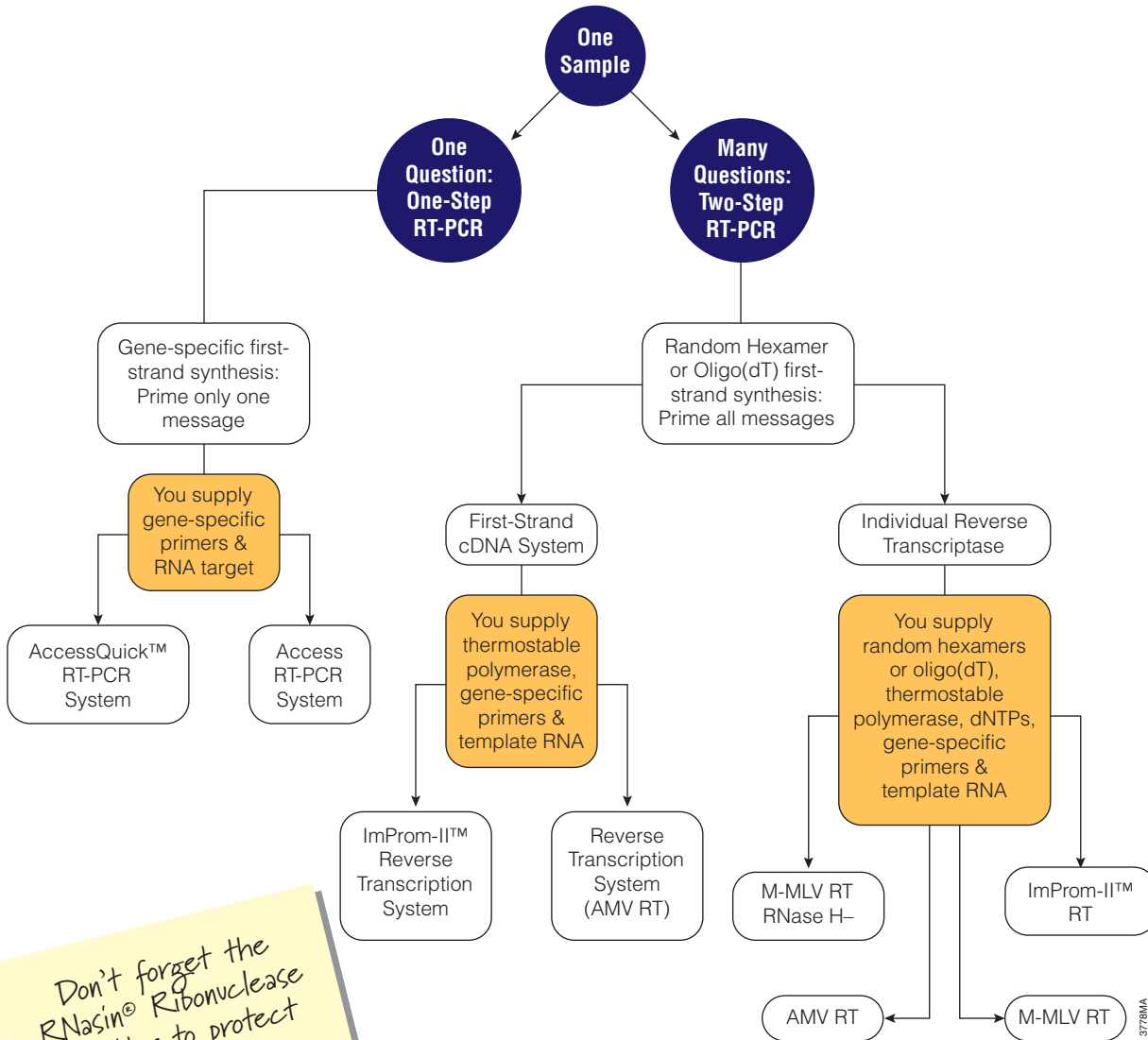


# Amplifying RNA with RT-PCR

## Overview

The use of reverse transcriptases to perform first-strand cDNA synthesis has had a huge impact on the study of biological processes. Finally, an RNA could be converted into DNA, allowing manipulation and cloning. Another important milestone in the evolution of molecular biology was the widespread application of

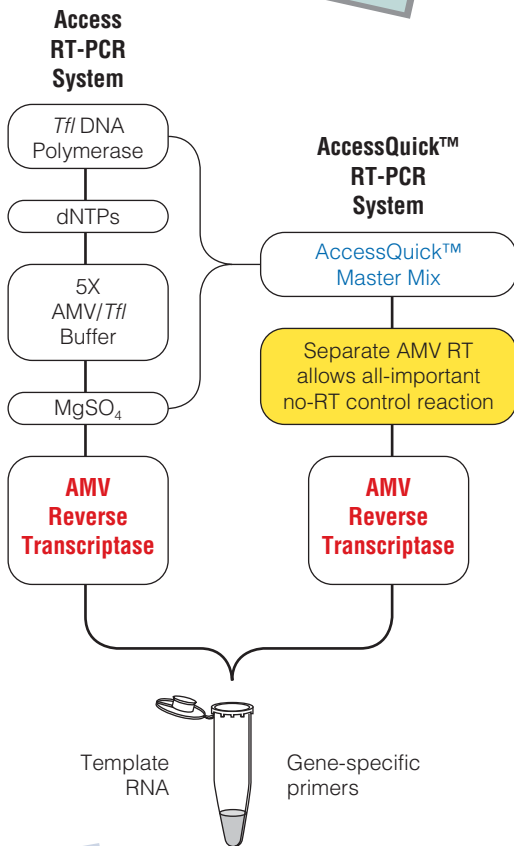
PCR to amplify DNA. It didn't take long before first-strand cDNA synthesis and PCR were connected and RT-PCR was born. Today, this technique is used almost as often as PCR. Many options are available to researchers, and questions must be answered before deciding which system to choose.



Don't forget the RNasin® Ribonuclease Inhibitor to protect the RNA!  
See page 3

# Amplifying RNA with RT-PCR

Access RT-PCR System  
 - Maximum Control  
 AccessQuick™ RT-PCR System  
 - Maximum Convenience



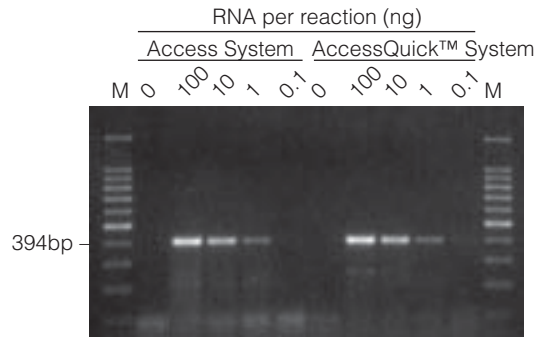
The downstream PCR primer primes first-strand cDNA synthesis.

AccessQuick™ RT-PCR System Protocol available at:  
[www.promega.com/tbs/9pia170/9pia170.html](http://www.promega.com/tbs/9pia170/9pia170.html)  
 Cat.# A1701, A1702, A1703

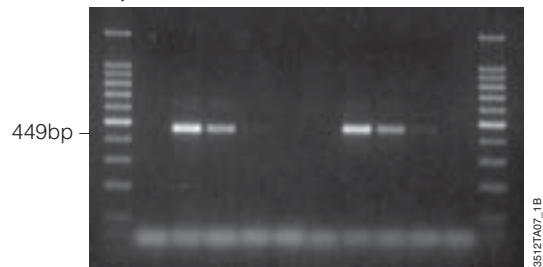
## AccessQuick™ and Access RT-PCR Systems

The Access and AccessQuick™ RT-PCR Systems offer the quickest route for amplifying a single, specific message. Both the Access and AccessQuick™ Systems are one-tube, one-step, coupled RT-PCR Systems. Combine the RNA and gene-specific primers with the kit components, and the entire reaction is ready. The Access System allows the most control over the reaction conditions, and the AccessQuick™ System offers the most convenience. Both use the robust AMV Reverse Transcriptase and the versatile Tfi DNA Polymerase along with a unique buffer that allows the most activity of both enzymes in the same reaction mixture. AMV RT is more heat-stable than M-MLV RT, thus allowing RT steps at temperatures of 42°C or higher. Tfi DNA Polymerase is indistinguishable from Taq DNA Polymerase in reaction characteristics. Like Taq DNA polymerase, Tfi DNA Polymerase leaves 3' A overhangs, allowing for direct cloning of amplicons with PCR cloning vectors like the pGEM®-T Easy Vector System.

### A. Human Protein Phosphatase I

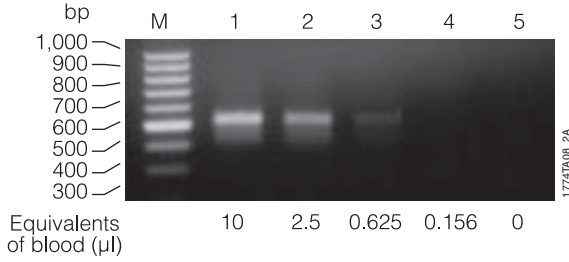


### B. γ-Actin



**Comparison of the Access and AccessQuick™ RT-PCR Systems.** Human protein phosphatase I (Panel A) and human γ-actin (Panel B) were amplified using the standard methods recommended with each system.

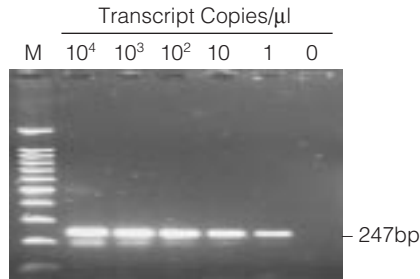
# Amplifying RNA with RT-PCR



mRNA captured on Streptavidin MagneSphere® Paramagnetic Particles (i.e., PolyATtract® Systems) can be used without elution in Access RT-PCR reactions.

**RT-PCR amplification of human β-actin from mRNA isolated from human blood.** mRNA was isolated from human blood using the PolyATtract® System 1000 mRNA Isolation System (1). Serial 4-fold dilutions of the RNA were prepared in Nuclease-Free Water. RT-PCR contained amounts of RNA equivalent to the indicated volume of starting blood, and primers complementary to the human β-actin transcript and were performed using the Access RT-PCR System as described (2). RNA samples used for RT-PCR were obtained from the final water resuspension of the Streptavidin MagneSphere® Paramagnetic Particles without magnetic capture, as the particles had no detrimental effect on RT-PCR. Reaction products (10μl) were resolved on a 1.5% agarose gel and visualized with ethidium bromide staining. Lane M contains 100bp DNA Ladder (Cat.# G2101).

## A. HAV



Access RT-PCR System Protocol available at:  
[www.promega.com/tbs/tb220/tb220.html](http://www.promega.com/tbs/tb220/tb220.html)  
Cat.# A1250, A1260, A1280  
Citations available at:  
[www.promega.com/citations/](http://www.promega.com/citations/)

## B. Astrovirus



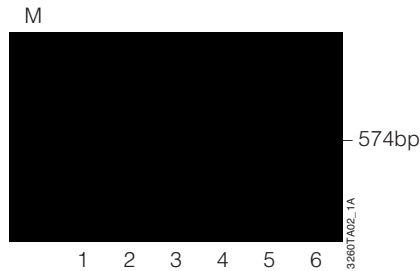
AccessQuick™ Master Mix can go through at least 10 freeze-thaw cycles with no decrease in performance.

## C. NLV I



AMV RT allows high-temperature RT reactions. Routinely, 45°C is recommended with the Access and AccessQuick™ RT-PCR Systems.

## D. NLV II



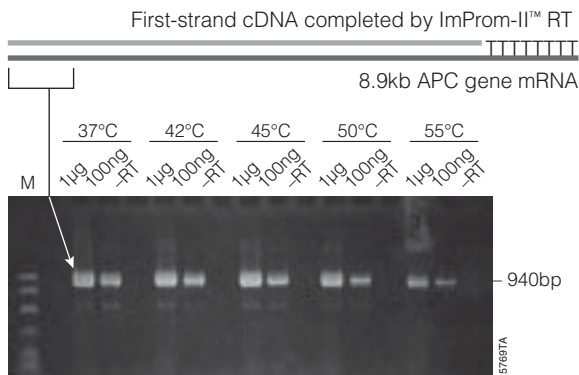
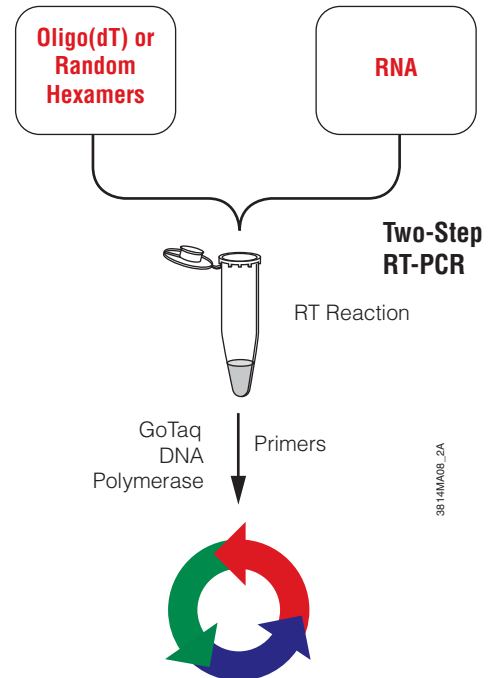
**Sensitivity of virus-specific RT-PCR assays using the Access RT-PCR System.** Identical serial dilutions of Hepatitis A Virus (HAV), Astrovirus, Norwalk-like Virus I (NLV I) and II (NLV II) transcripts were amplified using optimized virus-specific RT-PCR conditions (3). Five microliters of each RT-PCR product were then resolved on a 2% agarose gel. **Panel A.** HAV. **Panel B.** Astrovirus. **Panel C.** NLV I. **Panel D.** NLV II. Lanes 0, RT-PCR negative controls. Lanes M, 100bp DNA Ladder (Cat.# G2101).

Amplimers generated with either Access or AccessQuick™ RT-PCR Systems are directly compatible with the pGEM®-T Easy Vector System.

# Amplifying RNA with RT-PCR

## ImProm-II™ RT System & RT System

An RT reaction priming all mRNAs present is necessary if you need to analyze the messages from a number of different genes using one sample. Promega has two first-strand cDNA synthesis systems: the ImProm-II™ Reverse Transcription System and the Reverse Transcription System. In the ImProm-II™ System, ImProm-II™ RT and Buffer work together to generate full-length cDNAs, much like an RNase H–reverse transcriptase. First-strand cDNA is synthesized with either oligo(dT) or random hexamer priming. To have representation of the 5' end of the message, oligo(dT) priming requires that the reverse transcriptase make it all the way past where the primers bind. Random hexamer priming begins at multiple points along the template and makes several overlapping cDNAs. Elevated temperatures may be required to reverse transcribe RNA templates that contain secondary structure. Copies of the full amplicon are then made during the PCR step. The ImProm-II™ RT and ImProm-II™ Buffer support both RT and PCR. In fact, for maximum sensitivity, you can perform a 20µl RT reaction then add GoTaq® DNA Polymerase and primers in a minimal 2µl volume, and proceed to PCR amplification.



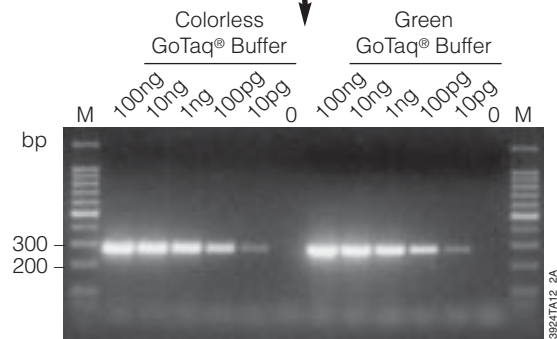
**Amplification of APC transcript dependent upon full-length, first-strand cDNA synthesis.** The indicated amount of HeLa cell total RNA was reverse transcribed with oligo(dT) using the ImProm-II™ Reverse Transcription System at the temperatures shown. Samples were first incubated for 10 minutes at 25°C before increasing the temperature for synthesis. The first-strand reaction was performed for 60 minutes, then the RT was heat-inactivated. The APC message (940bp) was amplified with 5 units of GoTaq® Flexi DNA Polymerase for 38 cycles. Full-length (8.9kb) cDNA synthesis is required for successful amplification. Complete details may be found in the Technical Manual (4). RNA was purified with PureYield™ RNA Midiprep System.

ImProm-II™ RT goes a long way! The entire 8.9kb APC gene must be made into cDNA to make the PCR product shown here.

# Amplifying RNA with RT-PCR

- ImProm-II™ RT can do one-step or two-step RT-PCR.
- The ImProm-II™ RT and ImProm-II™ Buffer **together** make for robust, full-length cDNA synthesis. **Do not** use a different buffer for the first-strand cDNA synthesis.
- The ImProm-II™ Buffer supports both PCR and RT reactions (see Uncoupled and Coupled methods below).
- One microliter of ImProm-II™ RT has the same cDNA synthesis activity as 200 units of M-MLV RT or 25 units AMV RT.
- When performing the one-step coupled RT-PCR reaction, always keep the ratio of ImProm-II™ RT to GoTaq or any *Taq* DNA Polymerase constant at 1µl ImProm-II™ RT:5 units of GoTaq® DNA Polymerase.

RT-PCR with GoTaq® DNA Polymerase works great!  
 The Green Reaction Buffer allows direct loading of agarose gels after the amplification. The Colorless Buffer allows direct fluorescent or absorbance measurements of samples without removal of the dyes.



**Detection of  $\beta$ -actin from total RNA.** Total RNA was isolated from Jurkat cells with the SV Total RNA Isolation System and the indicated amounts of the total RNA were reverse transcribed with the ImProm-II™ Reverse Transcription System. The cDNA for  $\beta$ -actin was amplified with GoTaq® DNA Polymerase with either the Colorless or Green Reaction Buffer. More details available in Glebs, A., Stencel, E. and Knoche, K. (2002) Introducing GoTaq® DNA Polymerase: Improved amplification with a choice of buffers. *Promega Notes* **83**, 21–24.

## Protocol: Reaction Options for Uncoupled PCR.

1. Add Promega reagents directly to the products of the reverse transcription reaction:

Component	Reactions		
	A Volume	B Volume	C Volume
Reverse Transcription Reaction	20µl	20µl	20µl
Nuclease-Free Water	18.6µl	NA	13µl
PCR Master Mix*	NA	NA	15µl
GoTaq® Flexi Buffer, 5X*	6.0µl	NA	NA
MgCl <sub>2</sub> , 25mM*	2.4µl	NA	NA
Gene-specific primer pair	2.0µl (25µM ea.)	1.0µl (20µM ea.)	2.0µl (50µM ea.)
GoTaq® DNA Polymerase (5u/µl)	1.0µl	1.0µl	NA
<b>Final PCR Volume</b>	<b>50µl</b>	<b>22µl</b>	<b>50µl</b>

2. Place in a thermal cycler and proceed with PCR.

For a detailed protocol, see Technical Manual #TM236.  
 N.A. Not applicable to this method.

\*Volumes must be adjusted to account for dNTP and MgCl<sub>2</sub> carryover from RT reaction.

## Protocol: Coupled RT-PCR.

1. Prepare Primers and Templates as described in Technical Manual #TM236.
2. For each 20µl RT-PCR volume, combine the components of the ImProm-II™ Reverse Transcription System and GoTaq® Flexi DNA Polymerase on ice.

Nuclease-Free Water	5.9µl
ImProm-II™ 5X Reaction Buffer	4.0µl
MgCl <sub>2</sub> , 25mM	1.6µl
dNTP Mix, 10mM	1.0µl
Recombinant RNasin® Ribonuclease Inhibitor	0.5µl (20u)
ImProm-II™ Reverse Transcriptase	1.0µl
GoTaq® DNA Polymerase (5u/µl)	1.0µl
Final volume per reaction	15µl

3. Add 5µl of Target + Primer Pair combination and overlay with nuclease-free mineral oil to prevent evaporation.
4. Incubate in a thermal cycler using the following protocol.

Anneal	25°C for 5 minutes
Extend first strand	42°C for 60 minutes
Heat inactivate	95°C for 5 minutes
PCR program	As appropriate
Final extension	As appropriate
Chill	4°C

# Amplifying RNA with RT-PCR

Example papers citing the use of the ImProm-II™ RT in real-time, quantitative RT-PCR:

## ImProm-II™ Reverse Transcriptase in qRT-PCR with SYBR® Green Detection

Behera, A.K., *et al.* (2005) Induction of host matrix metalloproteinases of *Borrelia burgdorferi* differs in human and murine lyme arthritis. *Infect. Immun.* **73**, 126–34.

Jazag, A., *et al.* (2005) Smad4 silencing in pancreatic cancer cell lines using stable RNA interference and gene expression profiles induced by transforming growth factor- $\beta$ . *Oncogene* **24**, 662–71.

Kim, M.S., Day, C.J. and Morrison, N.A. (2005) MCP-1 is induced by receptor activator of nuclear factor- $\kappa$ B ligand, promotes human osteoclast fusion, and rescues granulocyte macrophage colony-stimulating factor suppression of osteoclast formation. *J. Biol. Chem.* **280**, 16163–69.

Ohmae, T., *et al.* (2005) *Helicobacter pylori* activates NF- $\kappa$ B via the alternative pathway in B lymphocytes. *J. Immunol.* **175**, 7162–69.

Phelan, P.E., *et al.* (2005) Characterization of snakehead rhabdovirus infection in zebrafish (*Danio rerio*). *J. Virol.* **79**, 1842–52.

Ramunno, L., *et al.* (2005) Comparative analysis of gene sequence of goat CSN1S1 F and N alleles and characterization of CSN1S1 transcript variants in mammary gland. *Gene* **345**, 289–99.

Rider, C.V., *et al.* (2005) Stress signaling: Coregulation of hemoglobin and male sex determination through a terpenoid signaling pathway in a crustacean. *J. Exp. Biol.* **208**, 15–23.

Temes, E., *et al.* (2005) Activation HIF-prolyl hydroxylases by R59949, an inhibitor of the diacylglycerol kinase. *J. Biol. Chem.* **280**, 24238–44.

Xu, R.-H., *et al.* (2005) Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. *Nat. Methods* **2**, 185–90.

## ImProm-II™ Reverse Transcriptase in qRT-PCR with probe-based detection

Grivennikov, S.I., *et al.* (2005) Distinct and nonredundant in vivo functions of TNF produced by T cells and macrophages/neutrophils. Protective and deleterious effects. *Immunity* **22**, 93–104.

Wang, Y. and Jiang, H. (2005) Identification of a distal STAT5-binding DNA region that may mediate growth hormone regulation of insulin-like growth factor-I gene expression. *J. Biol. Chem.* **280**, 10955–63.

## Reverse Transcriptases

The Reverse Transcription System uses the higher temperature AMV Reverse Transcriptase at 42°C or beyond to work through tricky secondary structure. The system includes dNTPs, reaction buffers, control templates and control primers. All components are tested in RT-PCR applications.

Reverse Transcription System  
Cat.# A3500  
Protocol available at:  
[www.promega.com/tbs/tb099/tb099.html](http://www.promega.com/tbs/tb099/tb099.html)  
Citations available at:  
[www.promega.com/citations/](http://www.promega.com/citations/)

ImProm-II™ Reverse Transcription System  
Protocol available at:  
[www.promega.com/tbs/tm236/tm236.html](http://www.promega.com/tbs/tm236/tm236.html)  
Cat.# A3800  
ImProm-II™ Reverse Transcriptase available separately  
100 $\mu$ l (100 reactions) Cat.# A3802  
500 $\mu$ l (500 reactions) Cat.# A3803  
Citations available at:  
[www.promega.com/citations/](http://www.promega.com/citations/)

# Amplifying RNA with RT-PCR

## Reverse Transcriptases

For the most control and flexibility, build your own RT-PCR system from individual components. Start with a high-quality reverse transcriptase, add RT primers and dNTPs. Finally, choose your DNA polymerase.

Promega has a complete collection of reverse transcriptases to meet all your needs. For full-length cDNA synthesis, choose either M-MLV Reverse

Transcriptase, RNase H-, Point Mutant (Cat.# M3681), M-MLV Reverse Transcriptase, RNase H- (Deletion Mutant; Cat.# M5301) or ImProm-II™ Reverse Transcriptase. To get through tricky RNA secondary structure, choose the AMV Reverse Transcriptase (Cat.# M5101). For the most economical choice, M-MLV Reverse Transcriptase (Cat.# M1701) is suitable for many RT applications.

Works routinely at 37°C but can be used at temperatures up to 50°C.

Works routinely at 37°C but can be used at temperatures up to 60°C for two-step RT-PCR.

### Full Length cDNA

M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant  
Cat.#s M3681, M3682, M3683  
Citations available at: [www.promega.com/citations](http://www.promega.com/citations)

M-MLV Reverse Transcriptase, RNase H Minus (Deletion Mutant)  
Cat.# M5301  
Citations available at: [www.promega.com/citations](http://www.promega.com/citations)

ImProm-II™ Reverse Transcriptase  
Cat.# A3801, A3802, A3803  
Citations available at: [www.promega.com/citations](http://www.promega.com/citations)

### High Temperature Applications

AMV Reverse Transcriptase  
Cat.# M5101, M5108, M9004 (high concentration)  
Citations available at: [www.promega.com/citations](http://www.promega.com/citations)

### Most Economical

M-MLV Reverse Transcriptase  
Cat.# M1701, M1705  
Citations available at: [www.promega.com/citations](http://www.promega.com/citations)

The Point Mutant typically generates more cDNA than the deletion mutant.

ImProm-II™ RT and Buffer generate as much cDNA as SuperScript™-II.

Works routinely at 42°C but can be used at temperatures as high as 60°C.

Citations available at: [www.promega.com/citations/](http://www.promega.com/citations/)

# Amplifying RNA with RT-PCR

## Tips and Techniques

### Denaturation Prior to Reverse Transcription

We recommend a brief heat denaturation of any RNA template prior to reverse transcription or RT-PCR. This simple technique improves sensitivity.

1. Combine template RNA, RT primers and water in a minimal volume on ice (e.g., 5µl).
2. Transfer solution to a 70°C heat block for 5 minutes.
3. Transfer back to ice or ice/water bath for at least 5 minutes.
4. Quick-spin the tube prior to adding the remaining reagents for the RT or RT-PCR reaction.

### “Presynthesis” of Random Hexamer Primers

RT-PCR using random hexamer primers will not give optimal results if incubated at 37°C prior to the reverse transcription reaction, as 37°C may be too stringent of a temperature for hybridization of hexamers to the template. To remedy this, assemble the reaction on ice following the denaturation step, and then incubate at ambient temperature (22–25°C) for 10 minutes prior to bringing the reaction to 37°C. This room-temperature step allows the reverse transcriptase to extend the primer, and thus the primer will remain hybridized at the elevated temperature.

### Using RT Primers at Elevated Temperatures (>37°C)

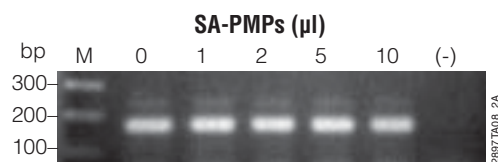
Performing reverse transcription reactions with random hexamers or oligo(dT) primers at temperatures above 37°C can give suboptimal results unless you first extend the primers somewhat at room temperature (22–25°C). Begin by assembling the RT reaction on ice, move the reaction to room temperature for 10 minutes and then move the reaction to the elevated temperature. The reverse transcriptase will extend the primer and allow the primer to remain hybridized at the elevated temperature. This approach is sometimes used to transcribe through troublesome secondary structure.

### Increase RT-PCR Sensitivity with RNase H

Treatment of reverse transcription reactions with RNase H after the reverse transcription step has been reported to increase sensitivity (5). Prior to the PCR step in two-step RT-PCR, add 1µl (2u) RNase H to the RT reaction and incubate for 20 minutes at 37°C.

### Amplify mRNA directly from PolyATtract® Magnetic Particles

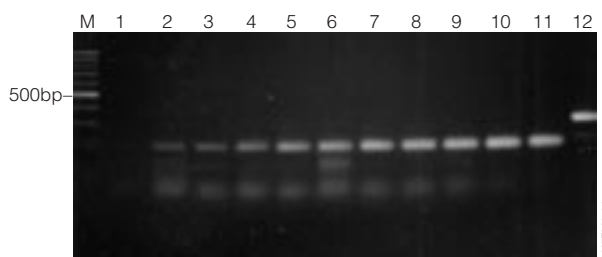
The PolyATtract® mRNA Isolation Systems and PolyATtract® System 1000 can be scaled to any reaction size as long as the recommended ratios are maintained. When working with small cell numbers, elution of the mRNA can be awkward. However, the Streptavidin MagneSphere® Paramagnetic Particles (SA-PMPs) do not interfere with RT-PCR. Add mRNA coated SA-PMPs directly to the RT-PCR.



**RT-PCR in the presence of SA-PMPs.** The indicated amount of SA-PMPs were added to a 50µl reaction containing 10ng mRNA from K562 cells. The reactions were amplified as detailed in Rhodes, R. and Kephart, D. (2000) Automated robotic isolation of Poly(A)+ mRNA using PolyATtract® mRNA Isolation Reagents. *Promega Notes* **75**, 10–12.

### Single-Cell RT-PCR using RNasin® Inhibitor

RNasin® Inhibitor has been used in applications where small cell numbers prevent the use of traditional RNA isolation methods prior to RT-PCR. Briefly, cells are lysed in a solution of RNasin® Inhibitor and Nuclease-Free Water using a freeze-thaw step. The entire solution is then used in RT-PCR analysis.



**RT-PCR analysis of bcr-abl transcript in K562 cells without RNA isolation.** Five microliters of cells were combined with 5µl of 2X RNasin® Ribonuclease Inhibitor Freeze Medium (2µl Recombinant RNasin® Ribonuclease Inhibitor [40u/µl] +18µl 0.15M NaCl/10mM Tris-HCl [pH 8.0]/5mM DTT). Lane M, 100bp DNA Ladder (predominant band is 500bp; Cat.# G2021); lanes 1–11 represent amplification signals from K562 cells numbering 0, 1, 5, 10, 20, 50, 100, 200, 500, 1,000 and 5,000, respectively. Lane 12, positive control RNA and primers supplied with the Access RT-PCR System (Cat.# A1250).

## References

1. Kephart, D. (1997) Rapid isolation of RNA from small quantities of human whole blood for use in RT-PCR analysis. *Promega Notes* **62**, 11–13.
2. *Access RT-PCR System Technical Bulletin* #TB220, Promega Corporation.
3. Legeay, O. *et al.* Detection of enteric pathogenic viruses in shellfish by RT-PCR. eNotes online: [www.promega.com/enotes/applications/ap0024\\_tabs.htm](http://www.promega.com/enotes/applications/ap0024_tabs.htm)
4. *ImProm-II™ Reverse Transcription System Technical Manual*, #TM236. Promega Corporation.
5. Nathan, M., Martz, L.M. and Fox, D.K. (1995) *Focus* **17**, 498.

# Amplifying RNA with RT-PCR

## Coupled, One-Step, Single-Tube

RT-PCR Systems	Size	Cat.#
Access RT-PCR System <sup>(g)</sup>	20 reactions	A1260
	100 reactions	A1250
	500 reactions	A1280
AccessQuick™ RT-PCR System <sup>(g)</sup>	20 reactions	A1701
	100 reactions	A1702
	500 reactions	A1703

For Laboratory Use.

## First-Strand Synthesis Systems:

### For Uncoupled, Two-Step

RT-PCR Systems	Size	Cat.#
ImProm-II™ Reverse Transcription System <sup>(a,b)</sup>	100 reactions	A3800
Reverse Transcription System <sup>(a)</sup>	100 reactions	A3500

For Laboratory Use.

## Separate Reverse

### Transcriptases

	Size	Cat.#
ImProm-II™ Reverse Transcriptase*	10 reactions	A3801
	100 reactions	A3802
	500 reactions	A3803
M-MLV Reverse Transcriptase, RNase H- Point Mutation**	2,500 units	M3681
	10,000 units	M3682
	50,000 units	M3683
M-MLV Reverse Transcriptase, RNase H-, (Deletion Mutant)	10,000 units	M5301
	10,000 units	M1701
M-MLV Reverse Transcriptase*	10,000 units	M1705
	300 units	M5101
	1,000 units	M5108
AMV Reverse Transcriptase* (Buffer with enzyme not directly compatible with PCR)	300 units	M5101
	600 units	M9004***

\*For Laboratory Use.

\*\*Only available in certain countries. Please inquire.

\*\*\*High Concentration.

## RT Accessory Products

	Size	Cat.#
Oligo(dT) <sub>15</sub> Primer	20µg	C1101
Random Primers*	20µg	C1181
Set of 4 dNTPs*	25µmol each	U1420
	40µmol each	U1240
Reverse Transcription 10X Buffer* (for use with AMV RT; compatible with PCR)	1.4ml	A3561
Magnesium Chloride Solution, 25mM*	1.5ml	A3511
Ribonuclease H*	50 units	M4281
	250 units	M4285

\*For Laboratory Use.

## Thermostable DNA Polymerases

	Size	Cat.#
GoTaq® Green Master Mix <sup>(g)*</sup>	100 reactions	M7122
	(Catalog numbers vary for Europe.) 1,000 reactions	M7123
GoTaq® Flexi DNA Polymerase <sup>(g)*</sup>	100 units	M8291
	(Catalog numbers vary for Europe.) 500 units	M8295
	2,500 units	M8296
	5,000 units	M8297
	10,000 units	M8298
	GoTaq® DNA Polymerase <sup>(g)*</sup>	100 units
(Catalog numbers vary for Europe.)	500 units	M3005
	2,500 units	M3008
PCR Master Mix <sup>(g)*</sup>	100 reactions	M7502
	1,000 reactions	M7505
<i>Pfu</i> DNA Polymerase <sup>(g)**</sup>	100 units	M7741
	500 units	M7745

\*For Laboratory Use.

\*\*Only available in certain countries, please inquire.

## Cloning Systems

	Size	Cat.#
pGEM®-T Easy Vector System I <sup>(i,j)</sup> * (no competent cells)	20 reactions	A1360
pGEM®-T Easy Vector System II* (with JM109 Competent Cells)	20 reactions	A1380
pTARGET™ Mammalian Expression Vector System <sup>(i-k)</sup> (contains JM109 Competent Cells)	20 reactions	A1410

\*For Laboratory Use.