

# Reverse Transcription System

INSTRUCTIONS FOR USE OF PRODUCT A3500.

## Reverse Transcription System

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### 1. Description

AMV Reverse Transcriptase synthesizes single-stranded cDNA from total or poly(A)+ isolated RNA (1). The Reverse Transcription System<sup>(a)</sup> provides tested reagents to efficiently reverse transcribe poly(A)+ mRNA or total RNA in 15 minutes. A polyadenylated 1.2kb transcript is provided as a control template for the cDNA synthesis reaction. cDNA synthesized with the Reverse Transcription System can be used directly in PCR.

### Citation using the Reverse Transcription System

- Basgupta, P. *et al.* (2006) Nicotine inhibits apoptosis induced by chemotherapeutic drugs by up-regulating XIAP and survivin. *Proc. Natl. Acad. Sci. USA* **103**, 6332-7.  
RT-PCR was performed to map the subtypes of nicotinic acetylcholine receptors on A549 cells. cDNA was synthesized using the Reverse Transcription System. Northern blotting to assess XIAP and survivin expression was performed, and probes were labeled using the Prime-A-Gene<sup>®</sup> Labeling System. Apoptosis was assessed in nicotine-stimulated cells using a DeadEnd<sup>™</sup> TUNEL Assay.

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## 2. Product Components and Storage Conditions

Product	Size	Cat. #
Reverse Transcription System	100 reactions	A3500

For Laboratory Use. Each system contains sufficient reagents for 100 reactions, processing 1µg of RNA per reaction. Includes:

- 1,500u AMV Reverse Transcriptase (High Conc.)
- 2,500u Recombinant RNasin® Ribonuclease Inhibitor<sup>(a)</sup>
- 50µg Oligo(dT)<sub>15</sub> Primer (0.5µg/µl)
- 50µg Random Primers (0.5µg/µl)
- 5µg 1.2kb Kanamycin Positive Control RNA (0.5µg/µl), 10µl
- 320µl dNTP Mixture, 10mM
- 1.4ml Reverse Transcription 10X Buffer
- 1.2ml MgCl<sub>2</sub>, 25mM
- 13ml Nuclease-Free Water

**Storage Conditions:** Store all components at -20°C.

## 3. Reverse Transcription Protocol

### 3.A. Reverse Transcription Reaction

Reverse transcription may be primed with either Oligo(dT)<sub>15</sub> or Random Primers. Choose Oligo(dT)<sub>15</sub> when priming at the 3' poly(A) region is desired. Choose Random Primers when priming throughout the length of the RNA is desired. Oligo(dT)<sub>15</sub> is frequently used when cDNA will be used for cloning and RT-PCR. Random Primers are sometimes preferred for cDNA that will be used in RT-PCR, especially when the PCR primers target a region near the 5'-end of the RNA.

1. Place 1µg (2µl) of 1.2kb Kanamycin Positive Control RNA, poly(A)+ mRNA or total RNA in a microcentrifuge tube, and incubate at 70°C for 10 minutes. Centrifuge briefly in a microcentrifuge, then place on ice.

2. Prepare a 20µl reaction by adding the following reagents in the order listed (this reaction can be scaled up or down, depending on the amount of RNA):

Component	Amount
MgCl <sub>2</sub> , 25mM*	4µl
Reverse Transcription 10X Buffer	2µl
dNTP Mixture, 10mM	2µl
Recombinant RNasin® Ribonuclease Inhibitor	0.5µl
AMV Reverse Transcriptase (High Conc.)	15u
Oligo(dT) <sub>15</sub> Primer OR Random Primers	0.5µg
1.2kb Kanamycin Positive Control RNA (2µl) OR poly(A)+ mRNA OR total RNA	1µg
Nuclease-Free Water to a final volume of	20µl**

\*The suggested magnesium concentration may be optimized for any given sequence to achieve better yields.

\*\*Final concentration of reaction components: 5mM MgCl<sub>2</sub>; 1X Reverse Transcription Buffer (10mM Tris-HCl [pH 9.0 at 25°C]; 50mM KCl; 0.1% Triton® X-100); 1mM each dNTP; 1u/µl Recombinant RNasin® Ribonuclease Inhibitor; 15u/µg AMV Reverse Transcriptase (High Conc.); 0.5µg Oligo(dT)<sub>15</sub> Primer or Random Primers per microgram RNA; 50ng/µl 1.2kb Kanamycin Positive Control RNA, poly(A)+ mRNA or total RNA.

3. When using Oligo(dT)<sub>15</sub> Primer, incubate the reaction at 42°C for 15 minutes. When using Random Primers (random hexamers), incubate the reaction at room temperature for 10 minutes, then incubate at 42°C for 15 minutes. The additional incubation at room temperature allows extension of the primers so that they remain hybridized when the temperature is raised to 42°C.
 

**Note:** There are different temperature requirements for the reverse transcription reaction when using Random Primers than when using Oligo(dT)<sub>15</sub>.
4. Heat the sample at 95°C for 5 minutes, then incubate at 0-5°C for 5 minutes. This will inactivate the AMV Reverse Transcriptase and prevent it from binding to the cDNA. For second-strand cDNA synthesis or agarose gel analysis, first-strand cDNA product may be used. For PCR amplification, proceed to Section 3.B. Alternatively, store the first-strand cDNA at -20°C until use.

#### Notes:

1. Prior to setting up the reaction, dispense the following reagents into individual tubes for use as needed prior to adding the RNA: water, buffer, dNTPs, MgCl<sub>2</sub>, Recombinant RNasin® Ribonuclease Inhibitor and AMV Reverse Transcriptase. This results in fewer pipetting steps and improved accuracy.

### 3.A. Reverse Transcription Reaction (continued)

- Specific downstream primers (provided by the user) may be substituted for the Oligo(dT)<sub>15</sub> Primer or Random Primers. The concentration of a specific primer should be adjusted according to the type of reverse transcription being performed. For example, when a 24mer primer is hybridized to 1.0µg of control template RNA, 800ng (100pmol) is required. When the identical primer is hybridized to a specific RNA in a total RNA sample, as little as 120ng (15pmol) is required. Specific primers are typically 19-30 bases long.
- For longer and/or more abundant transcripts, incubate the cDNA synthesis reaction at 42°C for up to 60 minutes.
- In cDNA synthesis, significantly fewer units of AMV Reverse Transcriptase are needed relative to M-MLV Reverse Transcriptase (Cat.# M1701).
- The use of elevated reverse transcription reaction temperatures (45-50°C) has been found to overcome problems of RNA secondary structure (2).
- Serial tenfold dilutions of the 1.2kb Kanamycin Positive Control RNA provided with the system have been used in amplification. Using the procedures outlined above, as little as 2.5 attomoles of the Control RNA can be detected.

### 3.B. Dilution of the Reaction for PCR Amplification

- Dilute the first-strand cDNA synthesis reaction to 100µl with TE buffer or Nuclease-Free Water.
- Prepare a 100µl PCR amplification mix by combining the following reagents. Note that template-specific upstream and downstream primers must be added at this point.

**Note:** The amount of input cDNA should be scaled down for smaller volume amplification reactions

Component	Amount
first-strand cDNA reaction	10-20µl
dNTP Mixture, 10mM	1.8µl
MgCl <sub>2</sub> , 25mM*	7.5µl
Reverse Transcription 10X Buffer	9.8µl
upstream primer	50pmol
downstream primer	50pmol
Taq DNA polymerase	2.5 units
Nuclease-Free Water to a final volume of	100µl**

\*The suggested magnesium concentration may be optimized for any given sequence to achieve better yields.

\*\*Final concentration of reaction components: <10ng/µl first-strand cDNA reaction, 200µM dNTPs, 2mM MgCl<sub>2</sub> (with contribution from first-strand cDNA reaction), 1X Reverse Transcription Buffer (10mM Tris-HCl [pH 9.0 at 25°C], 50mM KCl, 0.1% Triton® X-100).

- Proceed to thermal cycling according to your own specific experiment.

### 4. Composition of Buffers and Solutions

Reverse Transcription 10X Buffer (provided)	Random Primers
100mM Tris-HCl (pH 9.0 at 25°C)	0.5µg/µl hexamer oligonucleotides
500mM KCl	
1% Triton® X-100	

### 5. References

- Goodman, H.M. and MacDonald, R.J. (1979) Cloning of hormone genes from a mixture of cDNA molecules. *Meth. Enzymol.* **68**, 75-90.
- Miller, K. and Storts, D.R. (1995) A sensitive single-tube, two-enzyme system for RT-PCR. *Promega Notes* **53**, 2-5.

### 6. Related Products

#### Reverse Transcription

Product	Size	Cat.#
Access RT-PCR System	100 reactions	A1250
	500 reactions	A1280
Access RT-PCR Introductory System	20 reactions	A1260
ImProm-II™ Reverse Transcription System	100 reactions	A3800
ImProm-II™ Reverse Transcriptase	10 reactions	A3801
	100 reactions	A3802
	500 reactions	A3803

For Laboratory Use.

#### PCR Product Purification

Product	Size	Cat.#
Wizard® PCR Preps DNA Purification System*	50 preps	A7170
Vac-Man® Laboratory Vacuum Manifold, 20-sample capacity	1 each	A7231
Vac-Man® Jr. Laboratory Vacuum Manifold, 2-sample capacity	1 each	A7660

\*For Laboratory Use.

## 6. Related Products (continued)

### Reagents and dNTPs

Product	Concentration	Size	Cat.#
GoTaq® Green Master Mix	2X	100 reactions	M7122
	2X	1,000 reactions	M7123

For Laboratory Use. Catalog numbers may be different in Europe. Premixed solution of GoTaq® DNA Polymerase, GoTaq® Green Reaction Buffer, dNTPs and Mg<sup>2+</sup>. One reaction refers to a 50µl reaction.

Product	Concentration	Size	Cat.#
GoTaq® Flexi DNA Polymerase	5u/µl	100u	M8291
	5u/µl	500u	M8295
	5u/µl	2,500u	M8296
	5u/µl	5,000u	M8297
	5u/µl	10,000u	M8298

For Laboratory Use. Catalog numbers may be different in Europe. Includes 5X Green GoTaq® Flexi Reaction Buffer, 5X Colorless GoTaq® Flexi Reaction Buffer and Magnesium Chloride Solution, 25mM. Reaction buffers are magnesium-free.

Product	Concentration	Size	Cat.#
GoTaq® DNA Polymerase	5u/µl	100u	M3001
	5u/µl	500u	M3005
	5u/µl	2,500u	M3008

For Laboratory Use. Catalog numbers may be different in Europe. Includes 5X Green GoTaq® Reaction Buffer and 5X Colorless GoTaq® Reaction Buffer. Both buffers provide a final concentration of 1.5mM MgCl<sub>2</sub>.

Product	Concentration	Size	Cat.#
<i>Tfi</i> DNA Polymerase	5	100u	M1941
	5	1,000u	M1945
<i>Tli</i> DNA Polymerase	3	50u	M7101
<i>Tth</i> DNA Polymerase	5	100u	M2101
	5	500u	M2105
AMV Reverse Transcriptase	5-10	300u	M5101
	5-10	1,000u	M5108
AMV Reverse Transcriptase (High Conc.)	20-25	600u	M9004
Recombinant RNasin® Ribonuclease Inhibitor	20-40	2,500u	N2511
	20-40	10,000u	N2515
RNasin® Ribonuclease Inhibitor*	20-40	2,500u	N2111
	20-40	10,000u	N2115

For Laboratory Use.

\*For Research Use Only.

Product	Size	Cat.#
PCR Nucleotide Mix, 10mM	200µl	C1141
	1,000µl	C1145
dATP, 100mM	40µmol	U1201
dCTP, 100mM	40µmol	U1221
dGTP, 100mM	40µmol	U1211
dTTP, 100mM	40µmol	U1231
dATP, dCTP, dGTP, dTTP, 100mM each	40µmol each	U1240
dATP, dCTP, dGTP, dTTP, 100mM each	10µmol each	U1330
Lambda DNA/ <i>Hind</i> III Markers	100µg	G1711
100bp DNA Ladder	250µl (50 lanes)	G2101

For Laboratory Use.

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