TECHNICAL BULLETIN

Reverse Transcription System

Instructions for use of Product
A3500

Revised 1/14
TB099

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 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.

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
- 50µg Oligo(dT)$_{15}$ 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 $MgCl_2$, 25mM
- 13ml Nuclease-Free Water

Storage Conditions: Store 1.2kb Kanamycin Positive Control RNA (C138A) at –70°C. Store all other components at –20°C.

3. Reverse Transcription Protocol

3.A. Reverse Transcription Reaction

Reverse transcription may be primed with either Oligo(dT)$_{15}$ or Random Primers. Choose Oligo(dT)$_{15}$ 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)$_{15}$ 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
$MgCl_2$, 25mM*	4µl
Reverse Transcription 10X Buffer	2µl
dNTP Mixture, 10mM	2µl
Recombinant RNasin® Ribonuclease Inhibitor	0.5µl
AMV Reverse Transcriptase (High Conc.)	15u
Oligo$(dT)_{15}$ 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 $MgCl_2$; 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$(dT)_{15}$ 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$(dT)_{15}$ 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$(dT)_{15}$.

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, $MgCl_2$, Recombinant RNasin® Ribonuclease Inhibitor and AMV Reverse Transcriptase. This results in fewer pipetting steps and improved accuracy.

3.A. Reverse Transcription Reaction (continued)

2. Specific downstream primers (provided by the user) may be substituted for the Oligo(dT)$_{15}$ 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.
3. For longer and/or more abundant transcripts, incubate the cDNA synthesis reaction at 42°C for up to 60 minutes.
4. In cDNA synthesis, significantly fewer units of AMV Reverse Transcriptase are needed relative to M-MLV Reverse Transcriptase (Cat.# M1701).
5. The use of elevated reverse transcription reaction temperatures (45–50°C) has been found to overcome problems of RNA secondary structure (2).
6. 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

1. Dilute the first-strand cDNA synthesis reaction to 100µl with TE buffer or Nuclease-Free Water.
2. 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
$MgCl_2$, 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 $MgCl_2$ (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).

3. Proceed to thermal cycling according to your specific experiment.

4. Composition of Buffers and Solutions

Reverse Transcription 10X Buffer (provided)

100mM Tris-HCl (pH 9.0 at 25°C)
500mM KCl
1% Triton® X-100

Random Primers

0.5µg/µl hexamer oligonucleotides

5. References

1. Goodman, H.M. and MacDonald, R.J. (1979) Cloning of hormone genes from a mixture of cDNA molecules. *Meth. Enzymol.* **68**, 75–90.

2. 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	Concentration	Size	Cat.#
GoScript™ Reverse Transcription System		50 reactions	A5000
		100 reactions	A5001
GoScript™ Reverse Transcriptase		100 reactions	A5003
		500 reactions	A5004
Access RT-PCR System		100 reactions	A1250
		500 reactions	A1280
Access RT-PCR Introductory System		20 reactions	A1260
AMV Reverse Transcriptase	10u/µl	300u	M5101
	10u/µl	1,000u	M5108
AMV Reverse Transcriptase (High Conc.)	20–25	600u	M9004

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

6. Related Products (continued)

Reagents and dNTPs

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

Catalog numbers may be different in Europe. Premixed solution of GoTaq® DNA Polymerase, GoTaq® Green Reaction Buffer, dNTPs and Mg^{2+}. 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

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

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 $MgCl_2$.

Product	Concentration	Size	Cat.#
Tfl DNA Polymerase	5u/µl	100u	M1941
	5u/µl	1,000u	M1945
Tth DNA Polymerase	5u/µl	100u	M2101
	5u/µl	500u	M2105
Recombinant RNasin® Ribonuclease Inhibitor	20–40u/µl	2,500u	N2511
	20–40u/µl	10,000u	N2515
RNasin® Ribonuclease Inhibitor	20–40u/µl	2,500u	N2111
	20–40u/µl	10,000u	N2115

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/Hind III Markers	100µg	G1711
100bp DNA Ladder	250µl (50 lanes)	G2101

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