



Promega

Technical Bulletin

Reverse Transcription System

INSTRUCTIONS FOR USE OF PRODUCT A3500.



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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^(a) 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^(a)
- 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 MgCl₂, 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 20 μ l 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* | 4 μ l |
| Reverse Transcription 10X Buffer | 2 μ l |
| dNTP Mixture, 10mM | 2 μ l |
| Recombinant RNasin® Ribonuclease Inhibitor | 0.5 μ l |
| AMV Reverse Transcriptase (High Conc.) | 15u |
| Oligo(dT) ₁₅ Primer OR Random Primers | 0.5 μ g |
| 1.2kb Kanamycin Positive Control RNA (2 μ l) OR poly(A)+ mRNA OR total RNA | 1 μ g |
| Nuclease-Free Water to a final volume of | 20 μ l** |

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

**Final concentration of reaction components: 5mM MgCl₂; 1X Reverse Transcription Buffer (10mM Tris-HCl [pH 9.0 at 25°C]; 50mM KCl; 0.1% Triton® X-100); 1mM each dNTP; 1u/ μ l Recombinant RNasin® Ribonuclease Inhibitor; 15u/ μ g AMV Reverse Transcriptase (High Conc.); 0.5 μ g Oligo(dT)₁₅ Primer or Random Primers per microgram RNA; 50ng/ μ l 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, MgCl₂, 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 |
| <i>Taq</i> 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 MgCl₂ (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) | Random Primers |
|--|--------------------------------------|
| 100mM Tris-HCl (pH 9.0 at 25°C) | 0.5µg/µl hexamer oligonucleotides |
| 500mM KCl | |
| 1% Triton® X-100 | |

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 | 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 | 5-10 | 300u | M5101 |
| | 5-10 | 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 Mg²⁺. 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 MgCl₂.

| Product | Concentration | Size | Cat.# |
|--|---------------|---------|-------|
| <i>Tfl</i> DNA Polymerase | 5 | 100u | M1941 |
| | 5 | 1,000u | M1945 |
| <i>Tth</i> DNA Polymerase | 5 | 100u | M2101 |
| | 5 | 500u | M2105 |
| 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 |

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

^(a)U.S. Pat. No. 5,552,302.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

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