



TransScript® Reverse Transcriptase [M-MLV, RNase H⁻]

Cat. No. AT101

Storage: at -20°C for two years

Concentration: 200 units/ μ l

Description

TransScript® Reverse Transcriptase is a recombinant M-MLV reverse transcriptase with deficient RNase H activity.

- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- Anchored Oligo(dT)₁₈ Primer for higher yield and more full length cDNA.
- cDNA up to 12 kb.

Applications

- First-strand cDNA synthesis
- Multiple copy and low copy gene detection

Unit Definition

One unit of TransScript® RT incorporates 1 nmol of deoxyribonucleotide into acid-precipitable material in 10 minutes at 37°C using Poly(A)/Oligo(dT) as template/primer.

5×TS RT Buffer

250 mM KCl, 15 mM MgCl₂, 100 mM Tris-HCl pH 8.4

Kit Contents

Component	AT101-02	AT101-03
TransScript® RT	10000 U	5×10000 U
5×TS RT Buffer	200 μ l	5×200 μ l
Anchored Oligo(dT) ₁₈ Primer (0.5 μ g/ μ l)	50 μ l	250 μ l

First-Strand cDNA synthesis reactions

1. Reaction Components

Component	Volume
Total RNA/mRNA	0.1 ng-5 μ g/10 pg-500 ng
Anchored Oligo(dT) ₁₈ Primer (0.5 μ g/ μ l) or Random Primer(N9) (0.1 μ g/ μ l)	1 μ l
or GSP	2 pmol
10 mM dNTPs	1 μ l
5×TS RT Buffer	4 μ l
Ribonuclease Inhibitor (50 units/ μ l)	0.5 μ l
TransScript® RT	1 μ l
RNase-free Water	to 20 μ l

Optional: for higher efficiency, suggest to mix RNA, primer and water first. Incubate the mixture at 65°C for 5 minutes, on ice for 2 minutes. Then add other components.

2. Incubation

- For anchored oligo(dT)₁₈ primer or GSP, incubate at 42°C for 30 minutes.
- For random primer, incubate at 25°C for 10 minutes, then at 42°C for 30 minutes.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

RT-PCR

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
2× <i>TransTaq</i> [®] HiFi PCR SuperMix II	25 μl	1×
Nuclease-free Water	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

94°C	2-5 min	} 30-35 cycles
94°C	30 sec	
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	

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