TransScript® II Reverse Transcriptase [M-MLV, RNase H$^-$]

Cat. No. AH101

Storage: at -20°C for one year

Description

TransScript® II Reverse Transcriptase is a recombinant M-MLV reverse transcriptase with deficient RNase H activity and increased thermostability. The enzyme is active at up to 55°C. It provides higher specificity, higher yield and more full-length cDNA products.

- Increased thermostability for more full-length cDNA products.
- Reaction temperature at 42°C-55°C.
- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- Anchored Oligo(dT)$_{20}$ Primer for higher yield and more full length cDNA.
- cDNA up to 15 kb.

Applications

- First-strand cDNA synthesis, cDNA library construction, 3' and 5' RACE
- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template

Unit Definition

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

10×TS II RT Buffer

500 mM KCl, 30 mM $MgCl_2$, 100 mM Tris-HCl pH 8.4

Kit Contents

Component	AH101-02
TransScript® II RT	10000 U
10×TS II RT Buffer	100 µl
Anchored Oligo(dT)$_{20}$ Primer (0.5 µg/µl)	50 µl

First-Strand cDNA synthesis

1. Reaction Components

Component	Volume
Total RNA/mRNA	50 ng-5 µg/5-500 ng
Anchored Oligo(dT)$_{20}$ Primer (0.5 µg/µl)	1 µl
or Random Primer(N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
10 mM dNTPs	1 µl
10×TS II RT Buffer	2 µl
Ribonuclease Inhibitor (50 units/µl)	0.5 µl
TransScript® II RT	1 µl
RNase-free Water	to 20 µl

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)$_{20}$ primer or GSP, incubate at 50°C for 30 minutes.

• For random primer, incubate at 25°C for 10 minutes, then at 50°C for 30 minutes.

• For GC-rich or complex secondary structure RNA template, incubate at 55°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×*TransTaq*® HiFi PCR SuperMix II	25 μl	1×
ddH_2O	Variable	-
Total volume	50 μl	-

Thermal cycling conditions

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

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