TransScript® II Reverse Transcriptase [M-MLV, RNaseH-] (High Temperature RT)

Please read the data sheet carefully prior to use.

Cat. No. AH101

Storage: at -20℃ for two years

Concentration: 200 units/μl

Description

TransScript® II Reverse Transcriptase is a high-temperature reverse transcriptase obtained by genetic modification of *TransScript*® RT with deficient RNase H activity and increased thermostability, expressed and purified in *E. coli*. The enzyme can synthesize the first-strand cDNA under the conditions of 42℃-55℃, which is conducive to opening the RNA secondary structure, and the optimal reaction temperature is 50℃. It featureshigh sensitivity, high specificity, high processivity (more full-length cDNA), high thermostability and long half-life.

Features

- High thermostability: Reaction temperature at 42℃-55℃.
- High specificity, highyield, high processivity.
- DeficientRNase H activity, avoiding degradation of the template RNA in the DNA/RNA hybrid in the first-strand cDNA synthesis reaction, thereby ensuring the yield and length of the first-strand cDNA.
- Anchored Oligo$(dT)_{20}$ is specifically designed to bind to the first base next to the 5' end of Poly(A) tail of mRNA to anchor the binding site, providing higher specificity and high efficiency for first-strand cDNA synthesis.
- Synthetic fragments up to 15kb.

Applications

- cDNA library construction, primer extension, 3’ and 5’ RACE
- Multiple copies and low copy gene detection
- GC-rich or complex secondary structure RNA template

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

Before use, please centrifuge all the components briefly.

First-Strand cDNA synthesis

1. Add

Component	Volume
Total RNA/mRNA	0.1 ng-5 μg/10 pg-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	Variable
Total volume	20 μl

2. Mix well gently

• For anchored oligo(dT)$_{20}$ primer or GSP, incubate at 50℃ for 30 minutes.

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

• For GC-rich or complex secondary structure RNA template, incubate at 55℃ for 30 minutes.

3. Incubate at 85℃ for 5 seconds to inactivate *TransScript*® II RT.

Recommended PCR Reaction Component and Conditions (50 μl reaction volume)

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×
Nuclease-free Water	Variable	-
Total volume	50 μl	-

PCR

94℃	2-5 min	
94℃	30 sec	30-40 cycles
50-60℃	30 sec	
72℃	1-2 kb/min	
72℃	5-10 min	

Notes

• Avoid RNase contamination.

• To ensure successful reverse transcription, use high-quality RNA templates.

• For complex RNA templates, or to obtain higher synthesis efficiency, it is recommended to mix RNA template and RNase-free Water well first, incubate at 65°C for 5 minutes, and put on ice for 2 minutes before adding other reaction components.

• Mixing all the reaction components in one step can complete most reverse transcription reactions. For complex RNA templates, or to obtain higher synthesis efficiency, it is recommended to add thermal incubation steps for the template and primers according to the instructions.

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