

TransScript® Uni Reverse Transcriptase [M-MLV, RNase H⁻](High Temperature RT)

Please read the manual carefully before use.

Cat. No. AU101

Storage: at -20°C for two years

Concentration: 200 units/μl

Description

TransScript® Uni Reverse Transcriptase is a reverse transcriptase with high thermal stability expressed and purified from *E. coli* after genetic modification and screening. It enables a broad range of reaction temperature (42°C-65°C) with ultra-high thermal stability and deficient RNase H activity. Its high reaction temperature is beneficial to open RNA secondary structure when synthesizing the first-strand cDNA, with the optimal reaction temperature of 50°C. It also has the characteristics of high sensitivity, high specificity, high yield (more full-length cDNA), High thermal stability and long half-life.

Features

- Ultra-high thermal stability: reaction temperature 42°C-65°C.
- High sensitivity, high specificity, high yield and high processivity.
- The unique design of Anchored Oligo (dT)₂₀ can anchor the first base next to the mRNA Poly (A)+5' end, and the binding site is anchored, with high specificity, ensuring the efficiency and success rate of first-strand cDNA synthesis.
- Deficient RNase H activity. The degradation of the template RNA in the DNA/RNA hybrid in the first-strand cDNA synthesis reaction is avoided, thereby ensuring the yield and length of the first-strand cDNA.
- Synthesis length can be up to 20kb.

Applications

- High-copy number and low-copy number gene detection.
- High GC or RNA template with complex secondary structure.
- cDNA library construction, primer extension, 3' and 5' RACE.

Kit Contents

Component	AU101-02
TransScript® Uni Reverse Transcriptase	10000 units
10×TS Uni RT Buffer	100 μl
Anchored Oligo(dT) ₂₀ Primer (0.5 μg/μl)	50 μl

Before use, please centrifuge each component briefly.

First-strand cDNA synthesis

1. Prepare reaction mix with the following components

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 (0.1 μg/μl)	1 μl
or GSP	2 pmol
10 mM dNTPs	1 μl
10×TS Uni RT Buffer	2 μl
Ribonuclease Inhibitor(50 units/μl)	0.5 μl
TransScript® Uni Reverse Transcriptase	1 μl
RNase-free Water	Variable
Total volume	20 μl

2. Mix well gently for reverse transcription

- If use Anchored Oligo (dT)₂₀ or GSP, incubate at 50°C for 30 minutes.



- If use Random Primer (N9), incubate at 25°C for 10 minutes, and then incubate at 50°C for 30 minutes.
- For GC-rich or RNA template with complex secondary structure, it is suggested to increase the reaction temperature accordingly ($\leq 65^{\circ}\text{C}$).

3. Incubate at 85°C for 5 seconds to inactivate *TransScript*[®] Uni Reverse Transcriptase

Recommended qPCR Reaction Component and condition (20 μl reaction volumes)

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	0.4 μl	0.2 μM
Reverse Primer (10 μM)	0.4 μl	0.2 μM
2 \times <i>PerfectStart</i> [®] Green qPCR SuperMix	10 μl	1 \times
Passive Reference Dye (50 \times) (optional)	0.4 μl	1 \times
Nuclease-free Water	Variable	-
Total volume	20 μl	-

qPCR (Three-step)

94 30 sec
 94 5 sec
 50-60 15 sec
 72 10 sec

} 40-45 cycles

Dissociation Stage

qPCR (Two-step)

94 30 sec
 94 5 sec
 60 30 sec

} 40-45 cycles

Dissociation Stage

For the ABI qPCR instrument, we suggest using the following exposure time (Fluorescent signals can be collected during the annealing or extension stage for three-step qPCR):

- ★ For ABI Prism7700/7900, set the exposure time to 30 seconds.
- ★ For ABI Prism7000/7300, set the exposure time to 31 seconds.
- ★ For ABI Prism7500, set the exposure time to 34 seconds.
- ★ For ABI ViiA7, set the exposure time to at least 19 seconds.

Three-step qPCR is more suitable for higher amplification efficiency assay.

Two-step qPCR is more suitable for higher specificity assay

Recommended RCR Reaction Component and condition (20 μl reaction volumes)

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	0.4 μl	0.2 μM
Reverse Primer (10 μM)	0.4 μl	0.2 μM
2 \times <i>TransTaq</i> [®] HiFi PCR SuperMix II	25 μl	1 \times
Nuclease-free Water	Variable	-
Total volume	50 μl	-

PCR

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

} 35-40 cycles

Notes

- Avoid RNase contamination.
- For complex RNA templates, or to obtain higher synthesis efficiency, it is recommended to mix RNA template and RNase-free Water well. 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.

FOR RESEARCH USE ONLY

