TransStart® IV Reverse Transcriptase [M-MLV, RNase H-] (Anti Inhibition High Temperature RT)

Please read the data sheet carefully prior to use.

Cat. No. AW101

Storage: at -20°C for two years

Description

TransStart® IV Reverse Transcriptase is an M-MLV reverse transcriptase with high thermostability expressed and purified from E. coli after genetic modification. It enables a board range reaction temperature (42°C-65°C) and good resistance to inhibitors, 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 fast reaction speed, high sensitivity, high specificity, high yield (more full-length cDNA), and long half-life.

Features

- Wide range of applications: severely degraded RNA can be used as a template for reverse transcription.
- Agnosticto inhibitors remaining in RNA.
- Fast reaction speed: only 10 minutes for reverse transcription.
- Strong synthesis ability: synthesis length up to 20kb.
- High thermal stability: reaction temperature 42°C-65°C.
- High sensitivity: high detection rate for trace RNA.

Applications

- High-copy number and low-copy number gene detection.
- High GC or complex secondary structure RNA template.
- For difficult-to-handle RNA sample: degraded RNA template and RNA template containing RT enzyme inhibitor.
- cDNA library construction, primer extension, 3' and 5' RACE.

Kit Contents

Component	AW101-02
TransScript® IV Reverse Transcriptase	10000 Units
10×TS IV RT Buffer	100 µl
Anchored Oligo (dT)20 Primer (0.5 µg/µl)	50 µl

Prior to use, please centrifuge each component briefly.

First-strand cDNA synthesis

1. Add

Component	Component
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 IV RT Buffer	2 µl
Ribonuclease Inhibitor	0.5 µl
TransScript® IV Reverse Transcriptase	1 µl
RNase-free Water	Variable
Total volume	20 µl

2. Mix well gently

- If use Anchored Oligo $(dT)_{20}$ or GSP, incubate at 50°C for 10 minutes.
- If use Random Primer (N9), incubate at 25°C for 10 minutes, and then incubate at 50°C for 10 minutes.
- For GC-rich or complex secondary structure RNA template, it is suggested to increase the reaction temperature to some extent (≤65°C).

3. Incubate at 85°C for 5 seconds to inactivate *TransStart*® IV Reverse Transcriptase

Reaction Component for qPCR (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×*PerfectStart*® Green PCR SuperMix	10 µl	1×
Passive Reference Dye (50×) (optional)	0.4 µl	1×
Nuclease-free Water	Variable	-
Total volume	20 µl	-

PCR (Three-step)

94°C 30 min
94°C 5 sec
50-60°C 15 sec★
72°C 10 sec★
40-45 cycles
Dissociation Stage

qPCR (Two-step)

94°C 30 min
94°C 5 sec
60°C 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 is 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

Reaction Component for PCR (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×*TransTaq*® HiFi PCR SuperMix II	25 µl	1×
Nuclease-free Water	Variable	-
Total volume	50 µl	-

PCR (Three-step)

94°C 2-5 min
94°C 30 sec
50-60°C 30 sec★
72°C 1-2kb/min sec★
35-40 cycles
72°C 5-10 min

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