TransScript® IV One-Step gDNA Removal and cDNA Synthesis SuperMix

Please read the manual carefully before use.

Cat. No. AW311

Storage: at -20°C for two years

Description

TransScript® IV Reverse Transcriptase is a modified M-MLV reverse transcriptase. The product uses RNA as a template to synthesize the first-strand cDNA, meanwhile, remove the remaining genomic DNA in the RNA template in the same reaction volume. After the reaction, it only needs to heat at 85°C for 5 seconds to inactivate *TransScript*® IV RT/RI Enzyme Mix and gDNA Remover at the same time. The optimal reaction temperature of the enzyme is 50 °C, and it has ultra thermal stability.

Features

- Wide range of applications: severely degraded RNA can be used as a template for reverse transcription.
- Remarkable inhibitor tolerance: Excellent resistance to inhibitors remaining in RNA.
- Fast reaction speed: only 10 minutes for reverse transcription and genomic DNA removal.
- High processivity: synthesis length can be up to 20 kb.
- High thermal stability: reaction temperature 42°C-65°C.
- High sensitivity: high detection rate for trace RNA.
- After the reaction, *TransScript*® IV RT/RI Enzyme Mix and gDNA Remover are inactivated at the same time. Compared with the traditional method of pretreating RNA with DNase I, it avoids the damage of RNA after treatment by DNase I.

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 inhibitors.
- cDNA library construction, primer extension, 3' and 5' RACE.

Kit Contents

Component	AW311-02 (50 rxns)	AW311-03 (100 rxns)
TransScript® IV RT/RI Enzyme Mix	50 µl	100 µl
gDNA Remover	50 µl	100 µl
2×TS IV Reaction Mix	500 µl	1 ml
Random Primer (N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo $(dT)_{20}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

Prior to 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 mMdNTPs	1 µl
10×TS IV RT Buffer	2
Ribonuclease Inhibitor	0.5 µl
TransScript® IV 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 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 *TransScript*® IV RT/RI Enzyme Mix and gDNA Remover.

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

qPCR (Three-step)

94℃	30 sec	
94℃	5 sec	40-45 cycles
50-60℃	15 sec★	
72℃	10 sec★	

Dissociation Stage

qPCR (Two-step)

94℃	30 sec	
94℃	5 sec	40-45 cycles
60℃	30 sec★	

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

Recommended PCR Reaction Component and condition (50 μl reaction volumes)

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 mim	
94℃	30 sec	
50-60℃	30 sec	35-40 cycles
72℃	1-2 kb/min	
72℃	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.

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