TransScript® First-Strand cDNA Synthesis SuperMix

Please read the manual carefully before use.

Cat. No. AT301

Version No. Version 2.0

Storage: at -20°C for two years

Description

The product uses RNA as a template to efficiently synthesize the first-strand cDNA by *TransScript*® RT/RI Enzyme Mix and 2×TS Reaction Mix. It is easy to operate and can reduce contamination risk.

Features

- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- The product obtained from 15 minutes reaction is used for qPCR; the product obtained from 30 minutes reaction is used for PCR.
- Anchored Oligo(dT)$_{18}$ Primer is specifically designed to bind to the first base next to mRNA Poly(A)$^+$ on the 5' end with high specificity, ensuring high efficiency and success rate of first-strand cDNA synthesis.
- Random Primer (N9) or Gene Specific Primer (GSP) can be used to synthesize the first-strand cDNA.
- cDNA up to 12 kb.

Application

Multiple copy and low copy gene detection.

Kit Contents

Component	AT301-02	AT301-03
TransScript® RT/RI Enzyme Mix	50 µl	100 µl
2×TS Reaction Mix	500 µl	1 ml
Random Primer(N9) (0.1 µg/µl)	50 µl	100 µl
Anchored Oligo(dT)$_{18}$ Primer (0.5 µg/µl)	50 µl	100 µl
RNase-free Water	500 µl	1 ml

Briefly spin each component before use.

First-Strand cDNA synthesis

1. Reaction components

Component	Volume
Total RNA/mRNA	0.1 ng-5 µg/10 pg-500 ng
Anchored Oligo(dT)$_{18}$(0.5 µg /µl)	1 µl
or Random Primer(N9) (0.1 µg/µl)	1 µl
or GSP	2 pmol
2×TS Reaction Mix	10 µl
TransScript® RT/RI Enzyme Mix	1 µl
RNase-free Water	Variable
Total volume	20 µl

2. Mix gently

• For anchored oligo(dT)$_{18}$ primer or GSP, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).

• For Random Primer (N9), incubate at 25°C for 10 minutes. After that, incubate at 42°C for 15 minutes (for qPCR) or incubate at 42°C for 30 minutes (for PCR).

3. Incubate at 85°C for 5 seconds to inactivate *TransScript*® RT/RI.

Recommended qPCR system and conditions (taking 20 μl reaction system as an example)

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 qPCR 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
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 ABI instruments, read time is as follows, and fluorescent signals can be collected during the annealing or extension stage in the three-step method.

★ For ABI Prism 7700/7900, set the read time to 30 seconds.

★ For ABI Prism 7000/7300, set the read time to 31 seconds.

★ For ABI Prism 7500, set the read time to 34 seconds.

★ For ABI ViiA 7, set the read time 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 system and conditions (taking 50 μl reaction system as an example)

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	35-40 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.
- If the product is used for qPCR, for some special genes, the incubation time at 42 °C can be 30 minutes to obtain better results.

For research use only, not for clinical diagnosis

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