TransScript® II All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal)

Please read the manual carefully before use

Cat. No. AH341

Storage: at -18℃ or below for two years

Description

The kit provides all the necessary components (*TransScript*® II RT, RNase Inhibitor, Anchored Oligo(dT)$_{20}$ Primer, Random Primer(N9), dNTPs, Buffer) for reverse transcription reaction. It is provided at 5× concentration and used by adding gDNA remover, RNA template and H_2O for efficient first-strand cDNA synthesis at 42℃-55℃. Simultaneously, residual genomic DNA from RNA template can be removed. 5 ×*TransScript*® II All-in-One No-RT Control SuperMix for qPCR is supplied to prepare no-reverse transcriptase(RT) control, which is used to assess if the template for the qPCR reaction is contaminated with genomic DNA. This product is capable of minimizing contamination during operation with a simple workflow.

The resulting cDNA is suitable for qPCR, not for regular PCR.

Highlights

- "All SuperMix" form: Simultaneous cDNA synthesis and genomic DNA removal by only adding gDNA remover, RNA template and H_2O.
- High thermal stability: Supports reverse transcription at temperatures ranging from 42°C to 55°C.
- High synthesis efficiency enabled by optimal ratio of Oligo(dT)$_{20}$ Primer to Random Primer (N9) and optimized composition of the SuperMix, ensuring same reverse transcription efficiency for RNA templates of different concentrations and high synthesis efficiency for short cDNA.
- qPCR ready cDNA in 15 minutes.
- High compatibility with qPCR reagents.

Applications

- High copy and low copy gene detection

Kit Contents

Components	AH341-01 (50 rxns)
5×*TransScript*® II All-in-One SuperMix for qPCR	200 µl
5×*TransScript*® II All-in-One No-RT Control SuperMix for qPCR	20 µl
gDNA Remover	50 µl
RNase-free Water	1 ml

Briefly spin each component before use.

Procedures

Genomic DNA removal and first-strand cDNA synthesis

1. Add reaction components according to the following table:

Component	**Volume**
Total RNA/mRNA	≤1 µg/ ≤100 ng
5×*TransScript*® II All-in-One SuperMix for qPCR	4 µl
gDNA Remover	1 µl
RNase-free Water	Variable
Total volume	20 µl

2. Mix well gently, and incubate at 50°C for 15 minutes.
   For GC-rich or complex secondary structure RNA template, incubate at 55°C for 15 minutes.
3. Incubate at 85°C for 5 seconds to inactivate *TransScript*® II RT/RI and gDNA Remover.

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

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

qPCR (two-step)

94°C	30 sec	
94°C	5 sec	40-45 cycles
60°C	30 sec*	
Dissociation Stage		

For ABI qPCR instrument, read time is as follows (Fluorescent signals can be collected during the annealing or extension stage for three-step qPCR):

* For ABI Prism® 7700/7900, the time to 30 seconds.
* For ABI Prism® 7000/7300, the time to 31 seconds.
* For ABI Prism® 7500, the time to 34 seconds.
* For ABI ViiA® 7, the time is at least 19 seconds.

Two-step qPCR is more suitable for higher specificity assay.

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

Passive Reference Dye

• Passive Reference Dye I (50×)

ABI Prism 7000/7300/7700/7900, ABI Step One, ABI Step One Plus, ABI 7900HT, ABI 7900HT Fast

• Passive Reference Dye II (50×)

ABI Prism 7500, ABI Prism 7500 Fast, ABI QuantStudio Dx/3/5, ABI QuantStudio 6/7/12K Flex, ABI ViiA 7, Stratagene Mx3000P/Mx3005P/Mx4000

• No Passive Reference Dye

Roche LightCycler 480, Roche Light Cycler 96, MJ Research Chromo4, MJ Research Opticon 2, Takara TP-800, Bio-Rad iCycler iQ, Bio-Rad iCycler iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Scientific Pikoreal 96, Qiagen Corbett Rotor-Gene 6000, Qiagen Corbett Rotor-Gene G, Qiagen Corbett Rotor-Gene Q, Qiagen Corbett Rotor-Gene 3000, Mastercycler ep realplex

Notes

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

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