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

Cat. No. AT341

Storage: at -20°C for two years

Description

The kit provides all the necessary components for cDNA synthesis from total RNA or mRNA. It is provided at 5× concentration and used at 1× concentration by adding gDNA remover, RNA and H_2O. Simultaneous genomic DNA removal and cDNA synthesis are performed. After cDNA synthesis, gDNA remover and reverse transcriptase are inactivated by heating at 85°C for 5 seconds. The resulting cDNA is suitable for qPCR, not for regular PCR.

Highlights

- Simultaneous genomic DNA removal and cDNA synthesis.
- The optimal ratio of oligo$(dT)_{18}$ primer to random primer(N9) for qPCR ready cDNA.
- qPCR ready cDNA in 15 minutes.
- cDNA up to 250 bp.

Application

Multiple copy and low copy gene detection

Kit Contents

Components	AT341-01 (50 rxns)	AT341-02 (100 rxns)	AT341-03 (500 rxns)
5×*TransScript*® All-in-One SuperMix for qPCR	200 µl	400 µl	5×400 µl
5×*TransScript*® All-in-One No-RT Control SuperMix for qPCR	20 µl	40 µl	200 µl
gDNA Remover	50 µl	100 µl	5×100 µl
RNase-free Water	1 ml	2×1 ml	2×5 ml

Procedures

Genomic DNA removal and first-strand cDNA synthesis

1. Reaction Components

Components	Volume
Total RNA/mRNA	*
5×*TransScript*® All-in-One SuperMix for qPCR	4 µl
gDNA Remover	1 µl
RNase-free Water	to 20 µl

*Total RNA≤1 µg, mRNA≤100 ng (for 20 µl reaction system)

Optional: for higher efficiency, suggest to mix RNA and water first. Incubate the mixture at 65°C for 5 minutes, on ice for 2 minutes. Then add other components.

2. Incubate at 42°C for 15 minutes.

3. Incubate at 85°C for 5 seconds to inactivate enzymes.

Reaction Components

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	-

Thermal cycling conditions

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

Fluorescent signals can be collected during the annealing or extension stage. For ABI qPCR instrument, we suggest using the following signal collecting time:

* 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 sensitivity assay.

Passive Reference Dye

• Passive Reference Dye I (50×)

ABI Prism® 7000/7300/7700/7900, ABI Step One®, ABI Step One Plus®

• Passive Reference Dye II (50×)

ABI Prism® 7500, ABI Prism® 7500 Fast, ABI Q6, ABI QuantStudio® 6/7 Flex, ABI ViiA® 7, Stratagene Mx3000®/Mx3005P®, Qiagen Corbett Rotor-Gene® 3000

• 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