

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

Cat. No. AH341

Storage: at -20°C for one year

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 H₂O. 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)₂₀ Primer to random primer(N9) for qPCR ready cDNA.
- qPCR ready cDNA in 15 minutes.
- cDNA up to 250 bp.

Applications

- Multiple copy and low copy gene detection
- GC-rich or complex secondary structure RNA template

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

Procedures

Genomic DNA removal and first-strand cDNA synthesis

1. Reaction Components

Components	Volume
Total RNA/mRNA	*
5×TransScript® II 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 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 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× <i>TransStart</i> [®] Tip/Top Green qPCR SuperMix	10 µl	1×
Passive Reference Dye (50×) (optional)	0.4 µl	1×
ddH ₂ O	Variable	-
Total Volume	20 µl	-

Thermal cycling conditions

94°C	30 sec		94°C	30 sec	
94°C	5 sec	} 40-50 cycles	94°C	5 sec	} 40-50 cycles
50-60°C	15 sec*		60°C	30 sec*	
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

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