

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

Cat. No. AH341

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<sub>2</sub>O. 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)<sub>20</sub> 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>PerfectStart</i> <sup>TM</sup> 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	} 40-50 cycles	94°C	30 sec	} 40-50 cycles
94°C	5 sec		94°C	5 sec	
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<sup>®</sup> 7700/7900, the time to 30 seconds.
- \* For ABI Prism<sup>®</sup> 7000/7300, the time to 31 seconds.
- \* For ABI Prism<sup>®</sup> 7500, the time to 34 seconds.
- \* For ABI ViiA<sup>®</sup> 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<sup>®</sup> 7000/7300/7700/7900, ABI Step One<sup>®</sup>, ABI Step One Plus<sup>®</sup>
- Passive Reference Dye II (50×)  
ABI Prism<sup>®</sup> 7500, ABI Prism<sup>®</sup> 7500 Fast, ABI Q6, ABI QuantStudio<sup>®</sup> 6/7 Flex, ABI ViiA<sup>®</sup> 7, Stratagene Mx3000<sup>®</sup>/Mx3005P<sup>®</sup>, Qiagen Corbett Rotor-Gene<sup>®</sup> 3000
- No Passive Reference Dye  
Roche LightCycler<sup>®</sup> 480, Roche Light Cycler<sup>®</sup> 96, MJ Research Chromo4<sup>®</sup>, MJ Research Opticon<sup>®</sup> 2, Takara TP-800<sup>®</sup>, Bio-Rad iCycler iQ<sup>®</sup>, Bio-Rad iCycler iQ5<sup>®</sup>, Bio-Rad CFX96<sup>®</sup>, Bio-Rad C1000<sup>®</sup> Thermal Cycler, Thermo Scientific Pikoreal<sup>®</sup>96, Qiagen Corbett Rotor-Gene<sup>®</sup> 6000, Qiagen Corbett Rotor-Gene<sup>®</sup> G, Qiagen Corbett Rotor-Gene<sup>®</sup> Q

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