TransScript® II Green Two-Step qRT-PCR SuperMix

Cat. No. AQ301

Storage: at -20°C in dark for two years

Description

TransScript® II Green Two-Step qRT-PCR SuperMix is a two-step fluorescent quantitative RT-PCR (qRT-PCR) system providing highly efficient synthesis and amplification. 5×*TransScript*® II All-in-One SuperMix for qPCR together with gDNA remover allows simultaneous first-strand cDNA synthesis and gDNA removal at 42℃-55℃. 5×*TransScript*® II All-in-One No-RT Control SuperMix for qPCR is supplied as a control test in absence of reverse transcriptase, which is used to judge whether the qPCR template comes from cDNA or not. *PerfectStart*™ Green qPCR SuperMix is included as a component for qPCR amplification.

Highlights

- Simultaneous genomic DNA removal and cDNA synthesis to minimize contamination.
- High amplification efficiency, specificity and sensitivity, ensured by *PerfectStart*™ Green qPCR SuperMix, leading to accurate data.
- Passive reference dyes compatible with different qPCR instruments (normalize the fluorescent signals between reactions).

Applications

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

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

Kit Contents

Component	AQ301-01
5×*TransScript*® All-in-One SuperMix for qPCR	200 µl
5×*TransScript*® All-in-One No-RT Control SuperMix for qPCR	20 µl
gDNA Remover	50 µl
2×*PerfectStart*™ Green qPCR SuperMix	3×1 ml
Passive Reference Dye (50×)	120 µl
RNase-free Water	1 ml

First-Strand cDNA Synthesis

1. Reaction Components

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	to 20 μl

2. Incubation 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 (20 μl)

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	-

Thermal cycling conditions (three-step)

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

Thermal cycling conditions (two-step)

94°C 30 sec
94°C 5 sec
60°C 30 sec* } 40-45 cycles
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.

FOR RESEARCH USE ONLY