TransScript® II Multiplex Probe One-Step qRT-PCR SuperMix UDG

Cat. No. AQ322

Storage: Store at -20°C for two years

Description

TransScript® II Multiplex Probe One-Step qRT-PCR SuperMix UDG is designed for one-step qRT-PCR with high sensitivity, high synthesis efficiency and high amplification efficiency. This kit firstly synthesizes first-strand cDNA with RNA as templates using reverse gene-specific primers, and then performs qPCR with the synthesized cDNA as templates using both forward and reverse gene-specific primers and fluorescent probes to achieve one step from reverse transcription to qPCR in a single tube. After special optimization, this kit enables consistently amplifying multiple targets in a single reaction ensuring multiplex one-step RT-qPCR. dUTP/ UDG is included in the kit to degrade dU-containing ssDNA and dsDNA, which can prevent cross contamination.

Features

- Use *TransScript*® II Multiplex Probe One-Step Enzyme Mix UDG, 2×*PerfectStart*® Multiplex Probe One-Step Reaction Mix to efficiently synthesize RNA into first-strand cDNA for qPCR. It is easy to operate and reduces the chance of contamination during operation.
- Using UDG enzyme and dUTP to effectively prevent cross-contamination of PCR products with accurate data.
- High sensitivity , high specificity and the data are accurate.

Applications

- Singleplex to 4-plex RT-qPCR detection.
- High-copy and low-copy gene detection.
- RNA templates with high GC content or complex secondary structure.
- Detection of RNA virus or trace amounts of RNA.

Kit Contents

Component	AQ322-01	AQ322-02
TransScript® II Multiplex Probe One-Step Enzyme Mix UDG	80 µl	320 µl
2×*PerfectStart*® Multiplex Probe One-Step Reaction Mix	1 ml	4×1 ml
Passive Reference Dye (50×)	40 µl	160 µl
RNase-free Water	1 ml	4×1 ml

Recommended qPCR Reaction Components and Conditions (20 µl, 3 targets)

Component	Volume	Final Concentration
RNA Template	1 pg-100 ng	as required
20×primer-probe mix 1	Variable	0.4 µM forward primer 1[a]
		0.4 µM reverse primer 1[a]
		0.1 µM probe 1[b]
20×primer-probe mix 2	Variable	0.4 µM forward primer 2[a]
		0.4 µM reverse primer 2[a]
		0.1 µM probe 2[b]
20×primer-probe mix 3	Variable	0.4 µM forward primer 3[a]
		0.4 µM reverse primer 3[a]
		0.1 µM probe 3[b]
2×*PerfectStart*® Multiplex Probe One-Step Reaction Mix	10 µl	1×
TransScript® II Multiplex Probe One-Step Enzyme Mix UDG	0.8 µl	-
Passive Reference Dye (50×) (optional)	0.4 µl	1×
RNase-free Water	Variable	-
Total volume	20 µl	-

a. For most cases, we recommend using the primer concentration of 0.4 μM to obtain the optimum result. But in some cases, adjusting the primer concentration in the range of 0.2-1.0 μM can improve the result.
b. The optimum result can be obtained by adjusting probe concentration in the range of 0.1-0.3 μM.

Thermal cycling conditions (two-step)

50°C 5 min
94°C 30 sec
94°C 5 sec
60°C 30 sec* } 40-45 cycles

For ABI qPCR instrument, we suggest using the following exposure time:

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

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

Note

• Avoid RNase contamination.
• Use high-quality, intact RNA templates to ensure the success of RT-PCR.
• Only gene-specific primers are compatible with this kit. Oligo(dT) or random primers cannot be used.
• The working concentration of the probe will affect Ct value. Please determine the optimum amounts of probes based on experimental results.

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