PerfectStart® Uni RT&qPCR Kit

Please read the manual carefully before use.

Cat. No. AUQ-01

Version No. Version 3.0

Storage: at -20˚C in the dark for two years

Description

PerfectStart® Uni RT-qPCR Kit is a two-step fluorescence quantitative PCR reagent known for its high synthesis efficiency and amplification efficiency. In the same reaction system, it efficiently synthesizes the first-strand cDNA while simultaneously removing genomic DNA, utilizing 5×All-in-One Reaction Mix for qPCR and *TransScript*® Uni All-in-One Enzyme Mix at temperatures ranging from 42°C to 65°C. Additionally, it comes with 20×No RT Control Mix, to prepare a control without reverse transcriptase, allowing the determination of whether the qPCR template originates from cDNA. The qPCR amplification is performed using *PerfectStart*® Green qPCR SuperMix.

Features

- Efficient synthesis of first-strand cDNA from RNA templates using 5×All-in-One Reaction Mix for qPCR and *TransScript*® Uni All-in-One Enzyme Mix, requiring only 5 minutes for reverse transcription. Simultaneously, genomic DNA is removed, ensuring simplicity of operation protocol and reducing the risk of contamination during the process.
- The qPCR amplification is conducted using *PerfectStart*® Green qPCR SuperMix, known for its high amplification efficiency, high specificity, excellent sensitivity and data accuracy.
- The kit comes with Universal Passive Reference Dye suitable for different instrument models. The Universal Passive Reference Dye can adjust the variations between tubes caused by PCR pipetting errors, thereby ensuring data accuracy.

Applications

- High copy, low copy gene detection.
- RNA templates with high GC content or complex secondary structures.

Kit Contents

Component	AUQ-01
5×All-in-One Reaction Mix for qPCR	400 µl
TransScript® Uni All-in-One Enzyme Mix	100 µl
20×No RT Control Mix	40 µl
2×*PerfectStart*® Green qPCR SuperMix	15×1 ml
Universal Passive Reference Dye (50×)	600 µl
RNase-free Water	2×1 ml
Nuclease-free Water	3×5 ml

First-strand cDNA synthesis and gDNA removal

1. Reverse transcription reaction system and No RT Control reaction system (optional)

Component	Volume (RT Reaction)	Volume (No RT Control)
Total RNA/mRNA	≤1 µg/ ≤100 ng	≤1 µg/ ≤100 ng
5×All-in-One Reaction Mix for qPCR	4 µl	4 µl
TransScript® Uni All-in-One Enzyme Mix	1 µl	-
20×No RT Control Mix	-	1 µl
RNase-free Water	Variable	Variable
Total volume	20 µl	20 µl

2. Gently mix and incubate at 50˚C for 5 minutes.
   For RNA templates with high GC content or complex secondary structures, consider raising the reaction temperature (≤65°C) appropriately.
3. Heat at 85°C for 2 minutes to inactivate *TransScript*® Uni RT/RI and gDNA Remover.

Recommended qPCR system and conditions (taking 20 µl reaction system as an example)

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×
Universal Passive Reference Dye (50×) (optional)	0.4 µl	1×
Nuclease-free Water	Variable	-
Total volume	20 µl	-

qPCR (three-step)

94℃ 30 sec
94℃ 5 sec
50-60℃ 15 sec★
72℃ 10 sec★
(94℃ 5 sec to 72℃ 10 sec: 40-45 cycles)
Dissociation Stage

qPCR (two-step)

94℃ 30 sec
94℃ 5 sec
60℃ 30 sec★
(94℃ 5 sec to 60℃ 30 sec: 40-45 cycles)
Dissociation Stage

For ABI instruments, the read time (either annealing or extension stage in the three-step method) is as follows:
★ For ABI Prism 7700/7900, set the read time to 30 seconds.
★ For ABI Prism 7000/7300, set the read time to 31 seconds.
★ For ABI Prism 7500, set the read time to 34 seconds.
★ For ABI ViiA 7, set the read time to at least 19 seconds.

Three-step qPCR is more suitable for higher amplification efficiency assay. Two-step qPCR is more suitable for higher specificity assay.

Instrument models suitable for Universal Passive Reference Dye

• Universal Passive Reference Dye
ABI Prism 7000/7300/7700/7900, ABI Step One, ABI Step One Plus, ABI 7900HT, ABI 7900HT Fast； 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

Notes

• For complex RNA templates or to achieve higher synthesis efficiency, it is recommended to mix the RNA template with RNase-free Water, incubate at 65˚C for 5 minutes, and put on ice for 2 minutes before adding other reaction components.
• Avoid RNase contamination.
• To ensure successful reverse transcription, use a high-quality RNA template.

For research use only, not for clinical diagnosis.

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