PerfectStart® III Fast Probe qPCR SuperMix UDG

Please read the manual carefully before use.

Cat. No. AQ722

Version No. Version 2.0

Storage: at -20°C for two years

Description

PerfectStart® III Fast Probe qPCR SuperMix UDG is supplied at a 2× concentration, and is a pre-mix reagent for the amplification and detection of DNA in probe-based qPCR. Simply add primers, probe, template, and Nuclease-free Water to perform singleplex, multiplex (up to quadruple) PCR reactions in one reaction well. The amount of fluorescence is proportional to the amount of amplified product during amplification, so the amount of nucleic acid in the sample can be determined by the detection of the amount of fluorescence. *PerfectStart*® Taq III Hot-Start DNA Polymerase(Genetically modified Taq DNA polymerase, using antibody blocking method to effectively block the activity of DNA polymerase to prevent non-specific amplification at low temperature), combined with optimal reaction buffer, which significantly improves template affinity, qPCR amplification performance and the sensitivity of low-concentration template detection. It supports full premixing of primers and probes, and has good impurity tolerance to low-purity nucleic acids (presence of inhibitors), which is suitable for a variety of detection scene. The dUTP/UDG in the SuperMix can function at room temperature to eliminate carry-over contamination caused by PCR products and ensure the accuracy of results.

Applications

Detection of DNA of animal, plant and microorganism by probe fluorescence quantitative PCR

Features

- Hot start Taq DNA Polymerase blocking by antibodies, enabling high specificity, high sensitivity, high amplification efficiency, and a wide range of applicable species.
- Specially optimized qPCR reaction buffer provides higher sensitivity and specificity.
- High-efficiency antibody blocking, with optimal qPCR reaction buffer, supports full premixing of primers and probes, and enhances anti-inhibition.
- The dUTP/UDG anti-contamination system is used to effectively prevent carry-over contamination of PCR products, ensuring the accuracy of results.
- Universal Passive Reference Dye for different instruments to correct differences in fluorescence detection between wells due to pipetting errors.

Kit Contents

Component	AQ722-01	AQ722-02	AQ722-03
2×*PerfectStart*® III Fast Probe qPCR SuperMix UDG	1 ml	5×1 ml	15×1 ml
Universal Passive Reference Dye (50×)	40 μl	200 μl	600 μl
Nuclease-free Water	1 ml	5 ml	3×5 ml

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

Component	Volume	Final Concentration
Template	4 μl	as required
Forward Primer（10 μM）	0.4 μl	0.2 μM
Reverse Primer（10 μM）	0.4 μl	0.2 μM
TaqMan Probe（10 μM）	0.4 μl	0.2 μM
2×*PerfectStart*® III Fast Probe qPCR SuperMix UDG	10 μl	1×
Universal Passive Reference Dye (50×)（optional）	0.4 μl	1×
Nuclease-free Water	Variable	
Total Volume		20 μl

* Note: Be sure to thaw thoroughly and mix well before use, avoid excessive bubbles caused by vigorously shaking, and the amount of each component in the reaction system can be adjusted according to the following principles:

- Primer concentration: If the system contains multiple pairs of primers, usually the final concentration of each primer is 0.2 μM, which can obtain a better amplification effect, and the primer concentration can also be adjusted within 0.1~0.5 μM according to the reaction results.
- Probe concentration: If the system contains multiple probes with different fluorescence signals, the final concentration of each probe is usually 0.2 μM, which can be adjusted within 50~250 nM.
- Template dilution: qPCR reaction sensitivity is extremely high, and the accuracy of template addition has a great impact on the final quantitative results, so it is recommended to dilute the template and add it to the reaction system; the amount of template added can be adjusted as needed.

Reaction Program (Two-Step)

1) Standard Procedure

Step	Temperature	Time	Cycle
Pre-denaturation	95°C	5 min	1
Denaturation	95°C	5 sec	40~45 cycles
Annealing/Extension	60°C	15 sec★	

2) Fast Procedure

Step	Temperature	Time	Cycle
Pre-denaturation	95°C	20 sec	1
Denaturation	95°C	1 sec	40~45 cycles
Annealing/Extension	60°C	1 - 15 sec★★	

★Note: Please confirm whether the real-time PCR instrument supports the extension time of 15 sec.

★★Note: The recommended annealing/extension time is 5 sec; the extension time can also be set from 1 - 15 sec according to actual needs.

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

For research use only, not for clinical diagnosis

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Service telephone +86-10-57815020
Service email complaints@transgen.com