# *PerfectStart*® IV Fast Probe qPCR SuperMix UDG

**Please read the manual carefully before use.**

**Cat. No.** AQ732

**Version No.** Version 1.0

**Storage:** at -20˚C for two years

## Description

*PerfectStart*® IV 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 and template to perform singleplex, duplex and multiplex PCR reactions in one reaction well. This product contains newly upgraded *PerfectStart*® IV Taq Hot-Start DNA Polymerase (Genetically modified next generation 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 has strong resistance to impurity samples, which is suitable for a variety of detection scene. This product can support fast amplification, enabling 45 cycles of PCR amplification to be completed in 35 minutes. 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.
- High-efficiency antibody blocking supports full premixing of primers and probes, high anti-inhibition ability. This reagent can be used for direct amplication from blood, tissue homogenate, swab and other samples.
- Supports fast amplification, enabling 45 cycles of PCR amplification to be completed in 35 minutes.
- 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 | AQ732-01 | AQ732-02 | AQ732-03 |
|---|---|---|---|
| 2×*PerfectStart*® IV 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 | 1pg~1 μg | 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*® IV Fast Probe qPCR SuperMix UDG | 10 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 for use; the amount of template added can be adjusted as needed, if the template is a cDNA stock solution, the template volume should not exceed 1/10 of the total volume.

Reaction Program (Two-Step)

1) Standard Procedure

| Step | Temperature | Time | cycle |
|---|---|---|---|
| Pre-denaturation | 95℃ | 5 min | 1 |
| Denaturation | 95℃ | 5 sec | 40~45 cycles |
| Annealing/Extension (Fluorescence Signal Acquisition) | 60℃ | 15 sec | |

2) Fast Procedure

| Step | Temperature | Time | cycle |
|---|---|---|---|
| Pre-denaturation | 95℃ | 20 sec | 1 |
| Denaturation | 95℃ | 1~5 sec | 40~45 cycles |
| Annealing/Extension (Fluorescence Signal Acquisition) | 60℃ | 1~10 sec | |

*Note: For the first use, pre-experiment is required to confirm if the real-time PCR instrument supports the fast program. The heating and cooling rates are different for different models, and it is recommended that the total amplification time of 45 cycles should not be less than 30 minutes.

**Instrument models suitable for 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/Mx 4000

**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.**

Version number: V1.0-202405

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