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SuperFastStar Universal Probe Mixture

Cat. No. : CW3356S (1 mL)
CW3356M (5 mL)

Storage Condition: -20±5 ℃, Avoid repeated freeze-thaw. For frequent use, store at 2-8 ℃.

Components

Component	CW3356S 1 mL	CW3356M 5 mL
2×SuperFastStar Universal Probe Mixture	1 mL	5 mL
ddH_2O	1 mL	5 mL

Introduction

SuperFastStar Universal Probe Mixture is a 2 × premixed reagent designed for probe-based real-time PCR, including SuperFastStar DNA Polymerase, PCR Buffer, dNTPs, Mg^{2+}, enhancers and stabilizers. The reaction system simply needs to add templates, primers, probes, and it's easy to operate. SuperFastStar DNA Polymerase is a dual-antibody modified hot-start DNA polymerase with a blocking rate of more than 95% at temperatures of 55 ℃ and below, which can effectively reduce unspecific amplification at low temperatures. With the unique PCR buffer system, the qPCR amplification efficiency is significantly improved. This product has the characteristics of strong specificity, high sensitivity and good stability. It is suitable for the single and multiplex probe amplification, which has good tolerance to blood, saliva, tissue and other samples, and is suitable for SNP detection, methylation detection, HPV detection and other fields.

Notes

1. Please mix gently upside down before use and avoid foaming. It should be used after a short centrifugation.
2. Avoid repeated freeze-thaw of this product, because it may degrade the performance. This product can be stored at -20±5 ℃ for long-term storage. If frequent use is required in the short term, it can be stored at 2-8 ℃.

Protocol

The following protocol is an example of conventional PCR reaction systems and reaction conditions, which should be improved and optimized according to the template, primer structure and target fragment size in practice.

1. PCR reaction system

Reagent	25 μL System	50 μL System	Final Concentration
2×SuperFastStar Universal Probe Mixture	12.5 μL	25 μL	1×
Forward Primer，10 μM	0.5 μL	1 μL	0.2 μM[1)]
Reverse Primer，10 μM	0.5 μL	1 μL	0.2 μM[1)]
Probe，10 μM	0.25 μL	0.5 μL	0.1 μM[2)]
Template DNA[3)]	X μL	X μL	
ddH_2O	to 25 μL	to 50 μL	

Note:1) Generally, a better result can be obtained with a primer concentration of 0.2 μM. 0.1-1.0 μM can be set as a reference.
2) The concentration of the probe used is related to the type of Real-time PCR instruments, probe types, and fluorescent label substances used. Please refer to the instrument manual or the specific use requirements of each fluorescent probe for concentration adjustment.
3) Generally, the amount of DNA template is 10-100 ng genomic DNA or 1-10 ng cDNA as reference. Because template contains different copies of the target gene in different species, the template can be gradient diluted to determine the optimal dosage.

2. PCR program

Procedure	Temperature	Time	
Pre-denaturation	95 ℃	2 min[1)]	
Denaturation	95 ℃	10 s	} 40-45 cycles
Annealing/Extension	60 ℃(depends on primers)	30 s[2)]	

Note:1) The DNA polymerase used in this product can be activated at 95 ℃ for 30 s, but the template type also affects the pre-denaturation time. It is recommended to pre-denaturate for 2 min. Pre-denaturation and denaturation time can be extended for complex templates.
2) It is recommended to use two-step PCR reaction program. If good experimental results are not obtained due to the use of primers with low Tm values, three-step PCR amplification can be attempted.