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SuperFastStar Probe Mixture

Catalog Number: CW3347

Product Introduction

SuperFastStar Probe Mixture is a master mix designed for real-time PCR using probe methods (TaqMan, Molecular Beacon, etc.). The core ingredient, SuperFastStar DNA Polymerase, is a dual-antibody modified hot-start DNA polymerase that restores DNA polymerase activity after heating at 95 ℃ for 5 seconds, with a buffer optimized for qPCR with a reaction concentration of 2×. The unique combination of qPCR buffer system and hot-start enzyme effectively inhibits the production of non-specific products and significantly improves the amplification efficiency of qPCR, which is very suitable for qPCR reactions with high specificity, high sensitivity, single and multiple amplification. It is only needed to add the template, primers, probes, which is easy to use.

Storage Condition: -20±5 ℃.

Transport Conditions: 2~8 ℃.

Components

Component	CW3347S 1 mL	CW3347M 5 mL
2×SuperFastStar Probe Mixture	1 mL	5 mL
ddH_2O	1 mL	5 mL

Protocol

The following examples show the conventional qPCR reaction system and reaction conditions, which should be improved and optimized according to the different templates, primer structures, and target fragment sizes.

1. qPCR reaction system

Reagent	25 μL System	50 μL System	Final Concentration
2×SuperFastStar 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	0.25 μL	0.5 μL	0.1 μM [2)]
Template DNA [3)]	X μL	X μL	<1 μg (total DNA)
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 reaction program

Steps	Temperature	Time	Cycle Number
Pre-Denaturation	95℃	5-60 s [1)]	1
Denaturation	95℃	5-15 s	40-45
Annealing/Extension	60℃	30 s [2)]	

Note:1) This product can be activated at 95℃ for 30 s. For rapid program, 5s can be chosen. Denaturation time can be extended to 3 min for complex templates.

2) It is recommended to use two-step PCR reaction procedure. If good experimental results are not obtained due to the use of primers with low Tm values, three-step PCR amplification can be attempted. Please set the annealing temperature within the range of 56 ℃ to 64 ℃ as a reference.

General Guidelines for Primer Design

1. Design the probe first, then design the primer.
2. Primer sequence and probe sequence must not overlap. The optimal primer length is 20 bases.
3. Keep GC content in the range of 20-80%.
4. Avoid running the same nucleotide. If duplication cannot be completely avoided, it must be ensured that the number of consecutive G bases is less than 4.
5. Make sure the last 5 nucleotides of the 3 'end contain at most two G and/or C bases.
6. If a suitable primer sequence cannot be found, it may be necessary to check the target sequence and then select another amplicon site or screen more sites.

Note: Tm should be maintained at 56 ℃ -64 ℃.

Related Products

Cat. No.	Product Name
CWY129	Magbead Blood DNA Kit
CW2298	Universal Genomic DNA Kit
CW0531	NuClean Plant Genomic DNA Kit
CWY105	Samples Preprocessing Kit for Methylation Test
CWY036	Cell-Free DNA Storage Tube (glass, 10 mL)
CWY041	Fecal DNA Storage Tube