



SupeStar Probe Mixture

Product Number: PCM47

Shipping and Storage

-20±5°C

Components

| Component | PCM47 | PCM47 |
|--------------------------|-------|-------|
| | 1ml | 5ml |
| 2×SupeStar Probe Mixture | 1ml | 5ml |
| ddH ₂ O | 1ml | 5ml |

Description

SupeStar Probe Mixture is a premix specifically designed for real-time fluorescence quantitative PCR using probe methods (TaqMan, Molecular Beacon, etc.). The core component FastStar DNA Polymerase is a dual antibody modified hot start DNA polymerase, which can be heated at 95°C for 5 seconds to restore DNA polymerase activity. It has many advantages such as strong specificity and high detection sensitivity, and is paired with the optimal buffer optimized for qPCR. The reaction solution concentration is 2×. The unique qPCR buffer system and hot start enzyme combination of this product effectively inhibit the production of non-specific products and significantly improve the amplification efficiency of qPCR. It is very suitable for qPCR reactions with high specificity, high sensitivity, single and multiple amplifications. All customers need to do is add templates, primers, and probes, making it easy to use.

Preparation and precautions before the experiment

1. Before use, please gently mix the product upside down after it has completely melted, and centrifuge briefly before use.
2. This product is recommended to be packaged in small portions. If frequent use is required in the short term, it can be stored at 2-8°C for 6 months.
3. Avoid repeated freeze-thaw of this product, as repeated freeze-thaw (≤ 20 freeze-thaw cycles) may cause a decrease in product performance.
4. This product can be stored at -20±5°C in dark for a long time.
5. When configuring the reaction system for this product, it is best to do so on an ultra clean platform or in a sterile environment.

Protocol

The following example is a PCR reaction system and reaction conditions for amplifying a 1kb fragment using human genomic DNA as a template. In actual operation, corresponding improvement and optimization should be carried out according to the different template, primer structure and target fragment size.

1. PCR reaction system

| Component | 25µL reaction system | 50µL reaction system | Final Concentration |
|----------------------------|----------------------|----------------------|---------------------|
| 2×SupeStar Probe Mixture | 12.5 µL | 25 µL | 1× |
| Forward Primer, 10 µM | 0.5 µL | 1 µL | 0.2µM ¹⁾ |
| Reverse Primer, 10 µM | 0.5 µL | 1 µL | 0.2µM ¹⁾ |
| Probe, 10 µM | 0.25 µL | 0.5 µL | 0.1µM ²⁾ |
| Template DNA ³⁾ | X µL | X µL | |
| ddH ₂ O | Up to 25 µL | Up to 50 µL | |

Note: 1) Typically, the primer concentration is 0.2µM can achieve good results, ranging from 0.1 to 1.0µM serves as a reference for setting the range.



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2)The concentration of the probe used is related to the fluorescent quantitative PCR instrument used, the type of probe, and the type of fluorescent labeling substance. Please refer to the instrument manual or the specific usage requirements of each fluorescent probe for concentration adjustment during actual use.

3)Usually, the amount of DNA templates is based on 10-100ng genomic DNA or 1-10ng cDNA. Due to the different copy numbers of target genes contained in templates of different species, gradient dilution can be performed on the templates to determine the optimal template usage.

2. PCR reaction condition

| Step | Temperature | Time | cycles |
|------------------|-------------|---------------------|---------|
| Predenaturation | 95°C | 5-60s ¹⁾ | 1 |
| Denaturation | 95°C | 5-15s | } 40-45 |
| Annealing/Extend | 60°C | 30s | |

Note:1)The initial denaturation of this product at 95°C for 30 seconds is sufficient to activate the enzyme; Complex templates can be extended to 3 minutes for denaturation.

2)It is recommended to use a two-step PCR reaction program. If good experimental results cannot be obtained due to the use of primers with lower T_m values, a three-step PCR amplification can be attempted. The annealing temperature should be set within the range of 56°C -64°C as a reference.