Fast Probe Mixture

Product Number: PCM99

Shipping and Storage

-20°C, if frequently used, can be stored at 2-8°C to avoid repeated freeze-thaw as much as possible.

Components

Component	PCM99 1ml	PCM99 5ml
2×Fast Probe Mixture	1ml	5×1ml
ddH_2O	1ml	5×1ml

Description

The Fast Probe Mixture is a premixed system specifically designed for real-time fluorescence quantitative PCR using probe methods (TaqMan, Molecular Beacon, etc.), with a concentration of 2×,Contains specific Taq DNA Polymerase, PCR Buffer, dNTPs, Mg^{2+}, as well as enhancers and stabilizers, making the operation simple and convenient.Mainly used for detecting genomic DNA target sequences and cDNA target sequences after RNA reverse transcription. The specific Taq DNA Polymerase contained in this product can effectively reduce non-specific amplification caused by non-specific binding of primers and templates or primer dimers under room temperature conditions. The activation of the enzyme only needs to be incubated at 95°C for 30 seconds, greatly shortening the reaction time of PCR.The unique combination of PCR buffer system and hot start enzyme effectively inhibits the production of non-specific products, significantly improving the amplification efficiency of PCR, with stronger fluorescence signal, higher sensitivity, and wider linear range.

Notes

1. Before use, please gently mix it upside down and avoid foaming as much as possible. After briefly centrifuging, use it.
2. Avoid repeated freeze-thaw of this product, as repeated freeze-thaw may cause a decrease in product performance. This product can be stored in a dark place at -20°C for long-term storage. If frequent use is required in the short term, it can be stored at 2-8°C.
3. ROX dye is used to correct the fluorescence signal error generated between wells in the quantitative PCR instrument, and this product does not contain ROX dye.

Protocol

The following examples are the conventional PCR reaction system and reaction conditions. In practical operation, corresponding improvements and optimizations should be made based on different templates, primer structures, and target fragment sizes.

1. PCR reaction system

Reagent	50μL	25μL	20μL	Final Conc.
2×Fast Probe Mixture	25μl	12.5μl	10μl	1×
Forward Primer,10 μM	1μl	0.5μl	0.4μl	0.2μM 1)
Reverse Primer,10 μM	1μl	0.5μl	0.4μl	0.2μM 1)
Probe2)	1μl	0.5μl	0.4μl	0.2μM
Template DNA3)	Xμl	Xμl	Xμl	
ddH_2O	up to 50μl	up to 25μl	up to 20μl	

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. In the case of low amplification efficiency, the concentration of primers can be increased; When

non-specific reactions occur, the concentration of primers can be reduced to optimize the reaction system.

2)The final 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 conditions

Step	Temperature	Time	
Pre denaturation	95°C	30 s	
Denaturation	95°C	10 s	40-45cycles
Annealing/Extension	58°C	20 s	

Note:1)The enzyme used in this product is activated under pre denaturation conditions of 95℃ and 30seconds.Under this condition, most templates can perform well in de chaining.For templates with high GC content and complex secondary structures, the pre denaturation time can be extended to 1 minute to fully unwind the initial template. If the high-temperature treatment time is too long, it will affect the enzyme activity; For simple templates, pre denaturation for 20 seconds can also be used, and the optimal pre denaturation time can be determined based on the template situation.

2)It is recommended to use a two-step PCR reaction program, and the annealing temperature should be set at 58-64℃ as a reference range. When non-specific reactions occur, the annealing temperature can be increased.If good experimental results cannot be obtained due to the use of primers with lower Tm values, a three-step PCR amplification method can be attempted. The annealing temperature should be set within the range of 56℃-64℃ as a reference.

3)The annealing extension time settings for several common instruments are shown in the table below: when using Roche, BioRad, Agilent, and fluorescence quantitative PCR instruments from companies such as Hongshi and Dongshenglong, please set it at 20 seconds.When using ABI 7000/7300/7500, please set it to 30 seconds. The annealing/extension time can be set according to the use of different models of instruments and templates. Please follow the requirements of the instrument user manual for experimental operations.