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Universal Fast Probe Mixture

Product Number: PCM356

Shipping and Storage

-20±5℃.If frequently used, it can be stored at 2-8 ℃ to avoid repeated freeze-thaw as much as possible.

Components

Component	PCM356 1ml	PCM356 5ml
2×Universal Fast Probe Mixture	1ml	5ml
ddH_2O	1ml	5ml

Description

The Universal Fast Probe Mixture is a real-time fluorescence quantitative PCR premix for probe method, with a reaction solution concentration of 2×, Contains FastStar DNA Polymerase, PCR buffer, dNTPs, Mg^{2+}, enhancers, and stabilizers, all you need to do is add templates, primers, and probes, making the operation simple.The Universal Fast Probe Mixture is a dual antibody modified hot start DNA polymerase with a polymerase activity blocking rate of over 95% at temperatures up to 55 ℃, effectively reducing non-specific amplification at low temperatures. Simultaneously, the combination of a unique PCR buffer system significantly improves the efficiency of qPCR amplification.

Note

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 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 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	25μL	50μL	Final Conc.
2×Universal Fast Probe Mixture	12.5 μL	25 μL	1×
Forward Primer, 10μM	0.5 μL	1 μL	0.2 μM [1)]
Reverse Primer, 10μM	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	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.

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 program:

Step	Temperature	Time	cycles
Pre denaturation	95℃	2min[1)]	1
Denaturation	95℃	10 s	40-45
Annealing/Extension	60℃（Depending on primer）	30 s[2)]	

Note:1)The raw enzyme used in this product can be activated at 95 ℃ in 30s, but the template type also affects the pre denaturation time. It is recommended to use 2 minutes of pre denaturation. Complex templates can extend the pre denaturation and denaturation time.

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 Tm values, a three-step PCR amplification can be attempted.