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Universal FastStart Probe Mixture(UNG)

Product Number: PCM80

Shipping and Storage

-20 ± 5℃. If frequent use is required, it can be stored at 2-8℃ to avoid repeated freezing and thawing.

Component

Component	PCM80
2×Universal FastStart Probe Mixture(UNG)	1mL
RNase-Free Water	1mL

Description

Universal FastStart Probe Mixture (UNG) is a 2 × qPCR premix suitable for fluorescence quantitative detection using probe method, including Taq DNA Polymerase、Uracil-N-Glycosylase, PCR Buffer, dNTPs, Mg^{2+}, K^{+}, Enhancers and stabilizers, etc. Taq DNA Polymerase is a double antibody blocking type hot start enzyme, with a polymerase activity blocking rate of over 95% at temperatures of 55℃ and below. It can effectively reduce non-specific amplification at low temperatures. The added Uracil-N-Glycosylase and dUTP anti fouling system can catalyze dsDNA and ssDNA containing uracil, release free uracil, and reduce cross contamination of amplification products. The unique PCR buffer system can significantly improve the efficiency of qPCR amplification and accurately detect within a dynamic range of up to 6 logarithmic levels.

This product is suitable for single and multiple amplification, with high detection sensitivity and good specificity. It can detect templates as low as single copy and supports direct amplification of oral swabs and low concentration blood samples. It has strong universality and is widely used in gene expression and virus detection. And the glycerol content of the premix is extremely low, which can be directly combined with freeze-drying protectants for freeze-drying.

Protocol

The following are examples of conventional PCR reaction systems and conditions. In practical operation, corresponding improvements and optimizations should be made based on the template, primer structure, and target fragment size.

1. PCR reaction system

Reagent	25μL system	50μL system	Final concentration
2×Universal FastStart Probe Mixture(UNG)	12.5μL	25μL	1×
Forward Primer, 10μM	0.5μL	1μL	0.2μM
Reverse Primer, 10μM	0.5μL	1μL	0.2μM
Probe, 10μM	0.25μL	0.5μL	0.1μM
Template DNA	XμL	XμL	
RNase-Free Water	up to 25μL	up to 50μL	

Note: Usually, a primer concentration of 0.2μM can yield good results, and a reference range of 0.1-1.0μM can be used for setting.

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

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

2. PCR reaction program

Step	Temperature	Time	Cycle

UNG digestion	37℃	2min	1
Pre denaturation	95℃	3min	1
Denaturation	95℃	10s	45cycles
Annealing/extension	60℃ (depending on primer)	30s	

Note: The raw enzyme used in this product can be activated at 95℃ for 30 seconds. For templates with high GC content and complex secondary structure, the pre denaturation time can be extended to 1-3 minutes.

It is recommended to use a two-step PCR reaction procedure. If good experimental results cannot be obtained due to the use of primers with low Tm values, a three-step PCR amplification can be attempted.

Note

1. Before use, please invert and gently mix up and down to avoid foaming, and centrifuge briefly before use.
2. Avoid repeated freezing and thawing of this product, as repeated freezing and thawing may cause a decrease in product performance. This product can be stored at -20 ± 5℃ for long-term preservation. If frequently used in the short term, it can be stored at 2-8℃.