HyperProbe Mixture

Catalog number: H666048 (5 ml)

Storage condition: -20℃, if need to use frequently, can be stored in 2-8℃, try to avoid repeated freezing and thawing.

Products Content:

Component	5 ml
2×HyperProbe Mixture	5 x 1 ml
ddH2O	5 x 1 ml

Products Introduction

HyperProbe Mixture is a premixed system for real-time fluorescence quantitative PCR by probe method (TaqMan, Molecular Beacon, etc.). The concentration is 2×, which contains a new engineered DNA enzyme, PCR Buffer, dNTPs, Mg2+, enhancers and stabilizers, and it is easy to operate. It is mainly used for the detection of genomic DNA target sequences and cDNA target sequences after RNA reverse transcription.

This product contains highly sensitive engineered DNA enzyme, which can effectively reduce the non-specific amplification caused by the non-specific binding of primers and templates or primer dimer at room temperature, and at the same time, greatly improve the detection sensitivity and amplification efficiency, and the activation of the enzyme only needs to be incubated at 95 ℃ for 30 s, which greatly shortens the reaction time of PCR. The optimized PCR buffer system and enzyme mixture effectively inhibit the generation of non-specific products, which can significantly improve the PCR amplification efficiency, stronger fluorescence signal and higher sensitivity.

caveat

1. Before use, mix gently by turning up and down, avoid foaming as much as possible, and use after brief centrifugation.

2. Avoid repeated freezing and thawing of the product, which may degrade the performance of the product. This product can be stored at -20℃ for long term storage and protected from light. If frequent use is required within a short period of time, it can be stored at 2-8℃.

3. ROX dye is used to correct the fluorescence signal error generated between the wells of the quantitative PCR instrument, ROX is not contained in this product.

If you need to match ROX dyes with your instrument, please contact your local sales office or call CombiSense customer service.

4006-222-360.

Usage

The following examples are conventional PCR reaction systems and conditions, which should be improved and optimized according to the template, primer structure and target fragment size.

PCR reaction system

reagents	50 μl system	25 μl system	20 μl system	final concentratio
2×HyperProbe Mixture	25 μl	12.5 μl	10 μl	1×
Forward Primer, 10 μM Reverse Primer, 10 μM Probe2)	1 μl 1 μl	0.5 μl 0.5 μl	0.4 μl 0.4 μl	0.2 μM1) 0.2 μM1)
Template DNA3)	X μl	X μl	X μl	
ddH2O	up to 50 μl	up to 25 μl	up to 20 μl	

Attention:

(1) Usually, a primer concentration of 0.2 M can give better results, and 0.1-1.0 μM can be used as a reference for setting the range. If the amplification efficiency is not high, the concentration of primer can be increased; if non-specific reaction occurs, the concentration of primer can be decreased to optimize the reaction system.

(2) The final concentration of the probe used is related to the fluorescence quantitative PCR instrument used, the type of probe, and the type of fluorescent labeling substance, please refer to the manual of the instrument or the specific requirements for the use of each fluorescent probe to adjust the concentration.

3) Usually the amount of DNA template is 10-100 ng of genomic DNA or 1-10 ng of cDNA as a reference, as templates of different species

The number of copies of the target gene contained in them varies, and a gradient dilution of the template can be performed to determine the optimal amount of template to use.

PCR reaction conditions

move	temp	timing	
premutability	95°C	30s1)	
denaturation	95°C	10 s	40-45 cycles
Annealing/Extension	58°C	20 s2)	40-45 cycles

ention:

(1) The enzyme used in this product is activated by pre-denaturation at 95° C for 30 s. Most of the templates can be deconvoluted well under this condition. Under this condition, most of the templates can be well unchained. For templates with high GC content and complex secondary structure, the pre-denaturation time can be extended to 1 minute, so that the starting template can be fully unchained, and if the high temperature treatment time is too long, it will affect the activity of the enzyme; for simple templates, pre-denaturation can also be used for 20 s, and the optimal pre-denaturation time can be determined according to the template situation.

(2) It is recommended to use two-step PCR program, the annealing temperature should be 58-64℃ as the reference range, and the annealing temperature can be increased when non-specific reaction occurs. If you can't get good results due to the use of primers with low Tm value, you can try three-step PCR amplification, and the annealing temperature should be set in the range of 56℃-64℃ as a reference. The annealing and extension times for several common instruments are shown in the following table: 20 s for Roche, BioRad, Agilent, Hongshi, Dongshenglong, etc. 30 s for ABI 7000/7300/7500. The annealing/extension times can be set according to the different types of instruments and templates, please follow the instructions of the instruments. The annealing/extension time can be set according to different models of instruments and templates, please follow the instruction manual of the instrument.