Fast Probe Mixture

Project number: F665768

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

Product content

Component	F665768-5ml
2×Fast Probe Mixture	5 x 1ml
50 x Low ROX	200 μl
ddH2O	5 x 1ml

Product Introduction

Fast Probe Mixture is a pre-mixed system for real-time fluorescence PCR by probe method (TaqMan, Molecular Beacon, etc.), with a concentration of 2×, including Fast Taq DNA Polymerase, PCR Buffer, dNTPs, Mg2+ and so on, which is easy and convenient to operate. It is mainly used for the detection of genomic DNA target sequence and cDNA target sequence after RNA reverse transcription. The Fast Taq DNA Polymerase contained in this product can effectively reduce the non-specific amplification generated by the non-specific binding of primers and templates or primer dimerization at room temperature, and the activation of the enzyme only needs to be incubated at 95 ℃ for 30 s. The whole PCR reaction process can save about 40 minutes compared with the ordinary reaction, which greatly shortens the reaction time of PCR. The combination of unique PCR buffer system and fast hot start enzyme effectively inhibits the generation of non-specific products and significantly improves the PCR amplification efficiency with stronger fluorescence signal, higher sensitivity and wider linear range. The product has a wide range of applications and can be used for both normal and rapid quantitative PCR programs.

ROX dye is used to correct the fluorescence signal error generated between wells of a quantitative PCR instrument, and is generally used in Real Time PCR amplifiers from ABI, Stratagene, and other companies. The excitation optics vary from instrument to instrument, so the concentration of ROX dye must be matched to the corresponding fluorescence quantitative PCR instrument.

Instruments that do not require ROX calibration:

Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96 and others.

Instruments requiring Low ROX calibration:

ABI Prism7500/7500 Fast, QuantStudio®3 System, QuantStudio®5 System, QuantStudio®6 Flex System, QuantStudio®7 Flex System, ViiA 7 system. Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, and more.

Instruments requiring High ROX calibration:

ABI Prism 7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus, and others.

matters needing attention

1. Before use, please 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 this product, repeated freezing and thawing may degrade the product performance. This product can be stored for

long term at -20℃, protected from light. If frequent use is required within a short period of time, it can be stored at 2-8℃.

Usage

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

1.PCR reaction system

reagents	50μl reaction system	final concentration
2×Fast Probe Mixture	25 μl	1×
Forward Primer, 10μM	1μl	0.2μM[1)]
Reverse Primer, 10μM	1μl	0.2μM[1)]
Probe, 10 μM	1μl	0.2μM[2)]
Template DNA	2μl[3)]	
50xLow ROX or High ROX (optional)[4)]	1μl	1×
ddH_2O	Up to 50μl	

Note: 1) Usually the primer concentration of 0.2μM can get better results, and 0.1-1.0μM can be used as a reference for setting the range. 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, so please refer to the instruction manual of the instrument or the specific requirements of the use of each fluorescent probe for the adjustment of the concentration in actual use.

(3) Usually the amount of DNA template is 10-100ng genomic DNA or 1-10ng cDNA as a reference. Since the templates of different species contain different copy numbers of target genes, the templates can be subjected to gradient dilution to determine the optimal amount of template to be used.

(4) The excitation optical system varies from instrument to instrument, choose to add 50×Low ROX or 50×High ROX according to the instrument using fluorescence quantification.

2. PCR reaction program:

A two-step PCR reaction program is recommended, and this program is set up using the ABI7500 Fluorescent Quantitative PCR Instrument as a reference.

Step	temperature	time	
Pre denaturation	95° C	30s[1)]	
denaturation	95° C	5s	35-40 cycles
Annealing/Extension[2)]	60° C	30s	

Note: 1) The enzyme used in this product must be pre-denatured at 95° C for 30s to achieve enzyme activation. 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 1-4 minutes in order to make the starting template fully unchained.

(2) It is recommended to use two-step PCR reaction program, if you do not get good experimental results due to the use of primers with lower Tm values, etc., you can try to carry out three-step PCR amplification, and the annealing temperature, please use the range of 56 ℃ - 64 ℃ as a setting reference.