GoldStar Probe Mixture

Project number: G665762

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

Product content

Component	G665762-5ml
2×GoldStar Probe Mixture	5 x 1ml
50 x Low ROX	200 μl
ddH2O	5 x 1ml

Product Introduction

GoldStar Probe Mixture is a premixed system for real-time fluorescence quantitative PCR by probe method (TaqMan, Molecular Beacon, etc.), with a concentration of 2×, containing GoldStar Taq DNA Polymerase, PCR Buffer, dNTPs and Mg2+, which is easy and convenient to operate. It is mainly used for genomic DNA target sequence and RNA reverse transcription post-cDNA target sequence detection, such as gene expression analysis, copy number analysis, SNP genotype analysis, etc. It is suitable for fluorescence quantification by different types of probe methods. GoldStar Taq DNA Polymerase is a chemically modified, new and highly efficient hot-start enzyme, which has no polymerase activity at room temperature and effectively avoids non-specific amplification caused by non-specific binding of primers and templates or primer dimerization at room temperature, and the activation of the enzyme must be incubated at 95℃ for 10 minutes. The unique combination of PCR buffer system and hot-start enzyme significantly improves the amplification efficiency of PCR with stronger fluorescent signal and higher sensitivity to detect single-copy templates. A wider linear range and more accurate quantification of target genes can be obtained with this product. It is suitable for all fluorescent quantitative PCR instruments that do not require ROX as a calibration dye.

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 it gently by turning it up and down, avoid foaming as much as possible, and use it after centrifugation for a short time.

2. Avoid repeated freezing and thawing of this product, repeated freezing and thawing may degrade the product performance. This product can be stored for a long time at -20℃, protected from light. If frequent use is required in the short term, 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×GoldStar Probe Mixture	25 μl	1×
Forward Primer, 10μM	1μl	0.2μM[1)]
Reverse Primer, 10μM	1μl	0.2μM[1)]
Probe, 10μM	1μl	0.2μM[2)]
Template DNA	2μl[3)]	
50 x Low 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 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 during the 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

Caution! The pre-denaturation reaction of this product must be completed at 95° C for 10 minutes!

Two-step PCR:

Steps	Temperature	Time	
Pre denaturation	95℃	10min[1)]	
Denaturation	95℃	15s	} 35-40 cycles
Annealing/Extension[2)]	60℃	1min	

Note: 1) The hot-start enzyme used in this product shall be activated by the enzyme under the condition of pre-denaturation 95℃ and 10min.

(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.