GoldStar Probe Mixture

Item No. G665832 (5 mL)
Storage condition: -20℃, if need to use frequently, can be stored in 2-8℃, try to avoid repeated freezing and thawing.

Product content

Component	G665832 5 ml
2×GoldStar Probe Mixture	5 x 1 ml
50 x Low ROX	/
50 x High ROX	/
ddH2O	5 x 1 ml

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 ×, which contains GoldStar Taq DNA Polymerase, PCR Buffer, dNTPs and Mg2+, and is simple and convenient to operate. It is mainly used for genomic DNA target sequence and RNA reverse transcription cDNA target sequence detection, such as gene expression analysis, copy number analysis, SNP genotype analysis, etc. It is suitable for fluorescence quantification of different types of probes. GoldStar Taq DNA Polymerase is a chemically modified, new and highly efficient hot-start enzyme, which has no polymerase activity at room temperature, effectively avoiding non-specific amplification caused by non-specific binding of primers and template 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 PCR amplification efficiency, stronger fluorescence signal, higher sensitivity, and detection of single-copy templates. The use of this product can obtain a wider linear range, more accurate quantification of the target gene. It is suitable for all 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 the quantitative PCR instrument, and is generally used in Real Time PCR amplifiers from ABI, Stratagene, etc. The excitation optics of different instruments are different, so the concentration of ROX dye must be matched to the corresponding fluorescence quantitative PCR instrument. The excitation optics are different for different instruments, so the concentration of ROX dye must be matched to the corresponding quantitative PCR instrument.
Instruments that do not require ROX calibration (CW0932): Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96 and others.
Instruments requiring Low ROX correction (CW2625): 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 others. System, ViiA 7 system, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, and others.
Instruments requiring High ROX calibration (CW2626): ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus, etc.

Caveat

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 the product, as repeated freezing and thawing may degrade the performance of the product. 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 are examples of conventional PCR reaction systems and conditions, which should be improved and optimized according to the template, primer structure and fragment size.

1. PCR reaction system

reagents	50 μl reaction system	final concentration
2×GoldStar Probe Mixture	25 μl	1 ×
Forward Primer, 10 μM	1 μl	0.2 μM[1)]
Reverse Primer, 10 μM	1 μl	0.2 μM[1)]
Probe, 10 μM	1 μl	0.2 μM[2)]

Template DNA	2 μl[3)]	/
50 x Low ROX or High ROX (optional)[4)]	1 μl	1 ×
ddH2O	up to 50 μl	/

Attention:

(1) Usually, better results can be obtained with a primer concentration of 0.2 μM, and 0.1-1.0 μM can be used as a reference for setting the range.

(2) The concentration of the probe used is related to the fluorescence quantitative PCR instrument used, the type of probe and the type of fluorescent labeling material, please refer to the instrument manual 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. Since the templates of different species contain different copy numbers of target genes, the templates can be diluted in a gradient 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:

move	temp	timing	/
premutability	95° C	10 min[1)]	/
denaturation	95° C	15 s	35-40 cycles
Annealing/Extension [2)]	60° C	1 min	35-40 cycles

Attention:

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

(2) It is recommended to use two-step PCR reaction program, if you can not get good results due to the use of primers with low Tm value, etc., you can try three-step PCR amplification, the annealing temperature should be set in the range of 56 ℃ - 64 ℃ as a reference.