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2. PCR reaction procedure

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

Two-step PCR:

Steps	Temperature	Time	
Predenaturation	95 °C	10 min	
Denaturation	95 °C	15 s	35-40 cycles
Annealing/extension	60 °C	1 min	

Note:
1) The hot start enzyme used in this product must be activated under the condition of pre-denaturation 95°C and 10 min.
2) It is recommended to use the two-step PCR reaction procedure, if the use of low Tm value of primers and other reasons, can not get good results, you can try to use the three-step PCR amplification, annealing temperature please 56°C-64°C range as a reference.

This product is for scientific research only, which shall not be used for clinical diagnosis or other purposes.

Version: 07/2023

GoldStar Probe Mixture

Cat. No. : CW0932M CW2625M CW2626M (5 mL)
Shipping and Storage : -20°C, if you need to use frequently, can be stored in 2-8°C, try to avoid repeated freeze-thaw.

Components

Component	CW0932M 5 mL	CW2625M 5 mL	CW2626M 5 mL
	5 mL	5 mL	5 mL
2xGoldStar Probe Mixture	5x1 mL	5x1 mL	5x1 mL
50xLOW ROX	-	200 μL	-
50xHigh ROX	-	-	200 μL
ddH_2O	5x1 mL	5x1 mL	5x1 mL

Principle

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

ROX dye is used to correct the fluorescence signal error generated between the holes of the quantitative PCR instrument. It is generally used in ABI, Stratagene and other companies' Real Time PCR amplifiers. The excitation optical system of different instruments is different, so the concentration of ROX dye must be matched with the corresponding fluorescence quantitative PCR instrument. Instruments that do not require ROX correction (CW0932) :

Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96 and other instruments that require Low ROX correction (CW2625) :

ABI Prism7500/7500 Fast, QuantStudio 3 System, QuantStudio5 System,QuantStudio 6 Flex System, QuantStudio 7 Flex System, ViiA 7 system,Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, etc. Instruments requiring High ROX correction (CW2626) :ABI Prism7000/730017700/7900, Eppendorf, ABI Step One/Step One Plus, etc.

Precautions

Mix gently upside down before use, try to avoid foaming, and use after a short centrifuge.

Avoid repeated freeze-thaw of this product, which may degrade the performance of the product. This product can be stored at -20°C for a long time.

Keep out of light. If you need to use it frequently in the short term, it can be stored at 2-8 °C.

Procedure

The following examples are the conventional PCR reaction system and reaction conditions. In actual operation, corresponding improvement and optimization should be carried out according to the different template, primer structure and the size of the target fragment.

1. PCR reaction system

Reagent	50 μL reaction system	Final concentration
2xGoldStar Probe Mixture	25 μL	1X
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 pM 2)
Template DNA	2 μL 3)	
50xLow ROX or High ROX (optional) 4)	1 μL	1X
ddH_2O	up to 50 μL	

Note:

1) Usually primer concentration of 0.2M can get better results, can be used in 0.1-1.0μM as a reference for the set 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 labeled substance. Please refer to the instruction manual of the instrument or the specific use requirements of each fluorescent probe to adjust the concentration.

3) Usually the amount of DNA template is 10-100 ng genomic DNA or 1-10 ng cDNA as reference. Because of the different copy number of the target gene contained in the template of different species, gradient dilution of the template can be carried out to determine the best template usage.

4) The excitation optical system of different instruments is different, and 50×Low RoX or 50× high ROX can be added according to the instrument using fluorescence quantification.