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2×HotStar Probe Mixture (UNG)

Product Number: PCM63

Shipping and Storage

-20℃. If frequently used, it can be stored at 2-8℃ to avoid repeated freeze-thaw as much as possible.

Components

Component	PCM63 5mL	PCM63L 5mL	PCM63H 5mL
2×HotStar Probe Mixture (UNG)	5×1mL	5×1mL	5×1mL
50×Low ROX	-	200μl	-
50×High ROX	-	-	200μl
ddH_2O	5×1mL	5×1mL	5×1mL

Description

2× HotStar Probe Mixture (UNG) is a premixed system specifically designed for real-time fluorescence quantitative PCR using probe methods (TaqMan, Molecular Beacon, etc.), with a concentration of 2×,Contains Golden Star Taq DNA Polymerase, PCR Buffer, dNTPs (dTTP is completely replaced by dUTP), UNG enzyme, and Mg^{2+}, making the operation simple and convenient.Mainly used for detecting genomic DNA target sequences and cDNA target sequences after RNA reverse transcription, such as gene expression analysis, copy number analysis, SNP genotype analysis, etc.This product uses the dUTP-UNG anti pollution system, and dUTP is added during the preparation process of the PCR reaction system, resulting in the formation of amplification products containing dU bases.And this product can be eliminated by UNG enzyme treatment in the PCR system before the next PCR reaction. This effectively removes residual contamination of PCR products and greatly reduces false positives caused by amplification product contamination.The pre denaturation step of UNG enzyme in the PCR cycle can be inactivated, so it will not affect the formation of new dU based PCR products.The Golden Star Taq DNA Polymerase contained in this product is a chemically modified, novel and highly efficient hot start enzyme that has no polymerase activity at room temperature, effectively avoiding non-specific amplification caused by non-specific binding of primers and templates or primer dimers at room temperature. 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 fluorescence signal and higher sensitivity, which can detect single copy templates. Using this product can obtain a wider linear range and more accurate quantification of the target gene.

ROX dye is used to correct the fluorescence signal errors generated from hole to hole in quantitative PCR instrument. It is generally used in ABI, Stratagene and other companies' Real Time PCR amplifiers. The excitation optical system varies from instrument to instrument, so the concentration of ROX dye must be matched with the corresponding fluorescence quantitative PCR instrument.

Instruments without ROX correction:Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96, etc.

Instruments requiring Low ROX correction: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, etc.

Instruments requiring High ROX calibration:ABI Prism7000/7300/770/7900, Eppendorf, ABI Step One/Step One Plus, etc.

Notes

1. Before use, please gently mix it upside down and avoid foaming as much as possible. After briefly centrifuging, use it.
2. Avoid repeated freeze-thaw of this product, as repeated freeze-thaw may cause a decrease in product performance. This product can be stored for a long time at -20℃, away from light. If frequent use is required in the short term, it can be stored at 2-8℃.

For Research Use Only

Protocol

The following examples are the conventional PCR reaction system and reaction conditions. In actual operation, corresponding improvements and optimizations should be made based on different templates, primer structures, and target fragment sizes.

1. PCR reaction system:

Reagent	50μL reaction system	Final Concentration
2×HotStar Probe Mixture (UNG)	25μl	1×
Forward Primer, 10 μM	1μl	0.2 μM [1)]
Reverse Primer, 10 μM	1μl	0.2 μM [1)]
Probe, 10 μM	1μl	0.2 μM [2)]
Template DNA[3)]	2μl[3)]	
50×Low ROX or High ROX(optional)[4)]	1μl	1×
ddH_2O	up to 50μl	

Note: 1) Generally, a primer concentration of 0.2μM can obtain better results, and the final concentration of 0.1-1.0μM can be used as a reference for the set 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. Please refer to the instrument manual or the specific usage requirements of each fluorescent probe for concentration adjustment during actual use.

3)Usually, the amount of DNA templates is based on 10-100ng genomic DNA or 1-10ng cDNA. Due to the different copy numbers of target genes contained in templates of different species, gradient dilution can be performed on the templates to determine the optimal 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.

2. PCR reaction conditions

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

Two-step PCR:

Step	Temperature	Time	
UNG enzyme digestion	37℃/50℃	2-10 min	
Pre denaturation	95℃	10 min [1)]	
denaturation	95℃	15 s	35-40cycles
Annealing/Extension[2)]	60℃	1 min	

Note:1)The hot start enzyme used in this product must be activated under pre denaturation conditions of 95℃ and 10 minutes.

2)It is recommended to use a two-step PCR reaction program. If good experimental results cannot be obtained due to the use of primers with lower Tm values, a three-step PCR amplification can be attempted.

Three-step PCR:

Step	Temperature	Time	
UNG enzyme digestion	37℃/50℃	2-10 min	
Pre denaturation	95℃	10 min	
denaturation	95℃	15 s	35-40cycles
Annealing	55℃-65℃	30s	
Extension	72℃	30s	