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Gold Multiplex PCR Mix

Cat. No. : CW2347S (1 mL)
CW2347M (5 mL)

Storage: -20 ℃. For frequent use, it can be stored at 2-8 ℃.

Components

Component	CW2347S 1 mL	CW2347M 5 mL
2×Gold Multiplex PCR Mix	1 mL	5×1 mL
ddH_2O	1 mL	5×1 mL

Principle

Gold Multiplex PCR Mix is a premix system composed of GoldStar DNA Polymerase, Mg^{2+}, dNTPs, PCR stabilizer and reinforcer. With this product, there is no need to carry out the complicated optimization process of PCR reaction conditions, and only a simple condition exploration is needed to easily perform multiplex PCR reactions. The GoldStar DNA Polymerase included in the product is a chemically modified hot-start polymerase. The chemically modified enzyme has no polymerase activity at room temperature and is effective in avoiding the nonspecific amplification caused by the nonspecific binding of primer and template or primer dimer at room temperature. The activation of the enzyme requires incubation at 95 ℃ for 10 min. The enzyme is combined with PCR enhancers that enhance the specificity of the reaction and a unique buffer system that effectively extend all the primers in the reaction system without additional optimization. This MasterMix also includes GC Enhancer, which helps to achieve efficient amplification of "difficult" templates, such as those with high GC content. Gold Multiplex PCR Mix is suitable for various types of multiple PCR reactions, such as microsatellite analysis, genotyping, and SNP detection.

Quality Control

No exogenous nuclease activity was detected. The PCR method detected no residual host DNA. There was no obvious change in activity when stored at 2-8 ℃ for 3 months.

Procedure

The following examples show the conventional reaction system and reaction conditions, which should be improved and optimized according to the different templates, primer structures, and target fragment sizes.

1. 1.PCR Reaction System

Reagent	50 μL Reaction system	Final Concentration
2×Gold Multiplex PCR Mix	25 μL	1×
Primer Mix, 10 μM each	1 μL	0.2 μM
Template DNA	appropriate amount	
ddH_2O	to 50 μL	

Note: When designing primers, the difference between the Tm of each primer should be minimized, and the difference should be controlled within 5℃. The final concentration of 0.05-0.2 μM can be set as a reference for setting range. The primer concentration can be increased when the amplification efficiency is not high, and the primer concentration can be reduced in the case of non-specific reactions, so that the reaction system can be optimized.

2. PCR reaction condition

Step	Temperature	Time	
Pre-denaturation	95 ℃	10 min	
Denaturation	95 ℃	30 s	30-40 cycles
Annealing	55-65 ℃	30 s	
Extension	72 ℃	1 kb/min	
Final Extension	72 ℃	5 min	

Note:

1) In general experiments, the annealing temperature is 5 ℃ lower than the melting temperature (Tm) of the amplification primer, and the annealing temperature should be appropriately reduced when the ideal amplification efficiency cannot be obtained. When nonspecific reactions occur, increase the annealing temperature to optimize reaction conditions.

2) The extension time should be set according to the size of the amplified fragment. The amplification efficiency of the GoldStar DNA Polymerase included in this product is 1-2 kb /min.

3) The number of cycles can be set according to the downstream application of the amplification product. If the number of cycles is too low, there is insufficient amplification. If the number of cycles is too high, the mismatch rate increases, leading to significant nonspecific background. Therefore, the number of cycles should be minimized on the premise of ensuring the yield of the product.

4) The product must be pre-denaturated at 95 ℃ for 10 min to activate the enzyme.