Tinzyme Co., Limited

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Multiplex PCR Mix

Product Number:PCM26

Shipping Condition

-20°C; For frequent uses, store at 2-8°C

Components

component	PCM26 1mL	PCM26 5ml
2×Multiplex PCR Mix	1mL	5×1mL
ddH_2O	1mL	5×1mL

Description

Multiplex PCR Mix is a premixed system composed of Golden Star Taq DNA Polymerase, Mg^{2+}, dNTPs, and PCR stabilizers and enhancers. Using this product requires no complicated optimization process for PCR reaction conditions, and multiple PCR reactions can be easily carried out with simple condition groping. Golden Star Taq DNA Polymerase contained in this product is a chemically modified hot starter enzyme that can effectively reduce non specific amplification caused by primers mismatch at the beginning of the PCR reaction. Activation of the enzyme requires incubation at 95 °C for 10 minutes. This enzyme is combined with a PCR enhancer that can improve reaction specificity and a unique buffer system, allowing all primers in the reaction system to be effectively extended without additional optimization. This MasterMix also includes GC Enhancer that helps achieve efficient amplification of "difficult" templates, such as those with high GC content. Multiplex PCR Mix is suitable for various types of multiple PCR reactions, such as microsatellite analysis, genotyping, and SNP detection etc.

Quality Control

No exogenous nuclease activity was detected; Can efficiently amplify various kinds of DNA templates; No apparent activity change after being stored at 2-8°C for one month.

Protocol

The following protocol is an example of conventional PCR reaction system and condition. The actual protocol should be improved and optimized based on the template, primer structure and the size of the target.

1. PCR reaction system:

Reagent	50μl	Final Conc
2×Multiplex PCR Mix	25μl	1×
Primer Mix,10μM each	1μl	0.2μM
Template DNA	Xμl	
ddH_2O	Up to 50μl	

Note:When designing primers, minimize the difference between Tm of each primer, and try to control the difference within 5 ℃. Please use the final concentration of 0.05-0.2 for each primer concentration μM is used as a reference for setting the range. When the amplification efficiency is not high, the concentration of primers can be increased; When non specific amplification occurs, the primer concentration can be reduced, thereby optimizing the reaction system

2. PCR reaction program:

Step	Temperature	Time
Initialization	95℃	10min

Denaturation	95℃	30s	
Annealing	55-65℃	30s	30-40cycles
Elongation	72℃	1kb/min	
Final elongation	72℃	5min	

Note:1) This product achieves enzyme activation under pre denaturation conditions of 95 ° C for 10 minutes.

2) Annealing: the annealing temperature should be the 3-5°C lower than the Tm of primer. If the ideal amplification efficiency cannot be obtained, the annealing temperature should be changed in a gradient to optimize. When non-specific reactions occur, the annealing temperature should be appropriately increased. Two-step PCR can be used for primers with high Tm.

3) Elongation: The extension time should be set according to the length of the amplified fragment and the complexity of the template. The amplification efficiency of the Golden Star Taq DNA Polymeraseis 1-2 kb/min.

4) Cycles: The number of cycles can be set based on the downstream applications of the PCR product. If the number is too low, the amount of PCR product is insufficient; if the number is high, the probability of mismatch and the non-specific background are increased. Therefore, the number of cycles should be reduced as much as possible yet ensuring the yield of the product.