Version: 12/2023

SuperPlus Multiplex PCR Mix

Cat. No. : CW3319S (1 mL)
Storage Condition: -20 °C, avoid repeated freeze-thaw

Components

Component	CW3319S 1 mL
4×SuperPlus Multiplex PCR Mix	1 mL
ddH_2O	1 mL

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 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.

3) PCR products are prone to aerosol contamination, which leads to problems such as inaccurate and low confidence in experimental results. It is recommended to physically isolate the preparation area of the PCR reaction system from the PCR reaction area, use special pipettes and other equipment, and clean each experimental area regularly (wipe and clean with 0.5% sodium hypochlorite or 10% bleach) to ensure the reliability of the experimental results.

Introduction

SuperPlus Multiplex PCR Mix is a 4×premix for all types of multiplex PCR, containing DNA polymerases, PCR buffer, dNTPs, Mg^{2+}, stabilizers and enhancers. This product can be amplified by adding primers and templates, which is simple and convenient to operate, reduces the probability of contamination, and improves the detection throughput and reproducibility.

SuperPlus Multiplex PCR Mix contains DNA polymerase, which is a genetically engineered recombinant enzyme with 5'→3' DNA polymerase activity and no 5'→3' exonuclease activity. The DNA polymerase is an antibody-modified hot-start enzyme, which can effectively reduce the non-specific amplification due to primer-template nonspecific binding or primer dimer formation under room temperature, and has the advantages of short activation time, strong amplification ability, high sensitivity and good stability. The unique combination of PCR buffer system and hot-start enzyme significantly improves the amplification efficiency of PCR, with higher sensitivity and stronger inhibitor tolerance.

SuperPlus Multiplex PCR Mix can be used in a wide range of applications for all types of multiplex PCR, such as amplicon library preparation, microsatellite analysis, genotyping, and SNP detection.

Precautions

1. Before use, gently mix upside down after the product is completely melted, and use after a short centrifugation.
2. Avoid repeated freeze-thaw of this product, which may reduce the performance of the product. This product can be placed at -20°C for long-term storage.

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 application, template, primer structure, size of the target and amplification effect.

1. PCR reaction system

Reagent	25 μL system	50 μL system	Final concentration
4×SuperPlus Multiplex PCR Mix	6.25 μL	12.5 μL	1×
5×Primer Mix	5 μL	10 μL	1×
Template DNA	X μL	X μL	
ddH_2O	Up to 25 μL	Up to 50 μL	

Note:When designing primers, the difference between the Tm of each primer should be reduced as much as possible, and the difference should be controlled within 5 °C as much as possible. For each primer concentration, please use the final concentration of 0.05-0.2 μM as the reference for setting the range. Primer concentration can be increased when amplification efficiency is not high, and can be reduced in the case of non-specific amplification, thus optimizing the reaction system. For optimal amplification, using primer mixtures after vortexing for 10 s is recommended.

2. PCR reaction procedure

Steps	Temperature	Time	Cycle Number
Pre-denaturation	95 °C	2 min	1
Denaturation	95 °C	10 s	30-40
Annealing	55-65 °C	30 s	
Extension	72 °C	1 kb/min	
Terminal extension	72 °C	5 min	1