

Tinzyme Co., Limited

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Multi plus PCR MasterMix

Product Number: PCM319

Shipping and Storage

-20°C, avoid repeated freeze-thaw as much as possible.

Components

	PCM319
Component	1ml
4×Multi plus PCR MasterMix	1ml
ddH ₂ O	1ml

Description

Multi plus PCR MasterMix is a premixed system suitable for various types of multiple PCR, with a concentration of $4\times$. This product contains components such as DNA polymerase, PCR buffer, dNTPs, Mg^{2+} , stabilizer and enhancer. It can be amplified by adding primers and templates, making the operation simple and convenient, reducing the risk of contamination, and improving detection flux and reproducibility.

The DNA polymerase contained in Multi plus PCR MasterMix is a genetically engineered recombinant enzyme with $5' \rightarrow 3'$ DNA polymerase activity and no $5' \rightarrow 3'$ exonuclease activity; DNA polymerase, modified by a new type of antibody, is an antibody modified hot start enzyme that can effectively reduce non-specific amplification generated by non-specific binding or dimerization of primers and templates at room temperature. It also has excellent characteristics such as 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.

Multi plus PCR MasterMix has a wide range of applications and is suitable for various types of multiple PCR, such as amplicon library construction, microsatellite analysis, genotyping, and SNP detection.

Notes

- 1. Before use, please gently mix the product upside down after it has completely melted, and centrifuge briefly before use.
- 2. Avoid repeated freeze-thaw of this product, as repeated freeze-thaw may cause a decrease in product performance. This product can be stored at -20°C for a long time.

Protocol

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

1. PCR reaction system

Reagent	25µL reaction system	50µL reaction system	Final Concentration
4×Multi plus PCR MasterMix	6.25µL	12.5µL	$1 \times$
5×Primer Mix	5μL	10µL	$1 \times$
Template DNA	XμL	XμL	
ddH ₂ O	Up to 25µL	Up to 50µL	

Note:When designing primers, the difference in Tm between each primer should be minimized as much as possible, and the difference should be controlled within 5°C as much as possible.Please use the final concentration of 0.05-0.2μM as a reference for setting the range for each primer concentration.In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific amplification occurs, the primer concentration can be

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reduced to optimize the reaction system. To achieve the optimal amplification effect, it is recommended to use the primer mixture after a brief 10 second vortex oscillation before centrifugation.

2. PCR reaction conditions

Step	Temperature	Time	cycles
Predenaturation	95°C	2 min	1
Denaturation	95℃	10 s	
Annealing	55-65°C	30 s	30-40
Extend	72°C	1 kb/min	
Final extension	72°C	5 min	1

Note:1)In general experiments, the annealing temperature is 5°C lower than the melting temperature Tm of the amplification primer, and when ideal amplification efficiency cannot be achieved, the annealing temperature should be appropriately reduced; When a non-specific reaction occurs, increase the annealing temperature to optimize the reaction conditions.

2)The number of cycles can be set based on the downstream application of the amplification product. If the number of cycles is too small, the amplification amount is insufficient; If there are too many cycles, the probability of mismatch will increase, and the non-specific background will be severe. So, while ensuring product yield, the number of cycles should be minimized as much as possible.

3)PCR products are prone to aerosol contamination, leading to inaccurate and unreliable experimental results. It is recommended to physically isolate the PCR reaction system preparation area and PCR reaction area, use specialized pipettes and other equipment, and regularly clean each experimental area (using 0.5% sodium hypochlorite or 10% bleach for wiping and cleaning) to ensure the reliability of the experimental results.