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# Multiplex Pro MasterMix

**Product Number: PCM14**

## Shipping and Storage

-20°C; try to avoid repeated freeze-thaw cycles.

## Components

| Component | PCM14<br>1ml |
|---|---|
| 2.5×Multiplex Pro MasterMix | 1ml |
| $ddH_2O$ | 1ml |

## Description

Multiplex Pro MasterMix is a premixed system suitable for various types of multiple PCR, with a concentration of 2.5×, contains DNA polymerase, PCR buffer, dNTPs, $Mg^{2+}$ and components such as stabilizers and enhancers, this product can be amplified by adding primers and templates. The operation is simple and convenient, reducing the probability of contamination, and improving detection flux and reproducibility.

The DNA polymerase contained in Multiplex Pro MasterMix is a genetically engineered recombinant enzyme with 5'→ 3' DNA polymerase activity and no 5'→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.

Multiplex Pro MasterMix has a wide range of applications and is suitable for various types of multiplex PCR, such as microsatellite analysis, amplification library construction, 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℃ for a long time.

## Protocol

1. PCR reaction system

Extraction of DNA amplification reaction system:

| Reagent | 25μL reaction system | 50μL reaction system | Final Concentration |
|---|---|---|---|
| 2.5×Multiplex Pro MasterMix | 10 μL | 20 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 in Tm between each primer should be minimized as much as possible, and the difference should be controlled within 5℃ 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 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 condition

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

Note:1)In general experiments, the annealing temperature is 5℃ 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.