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# 2.5×STR Master Mixture

**Product Number: PCM311**

## Shipping and Storage

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

## Components

| Component | PCM311<br>1 ml |
|---|---|
| 2.5×STR Master Mixture | 1 ml |
| $ddH_2O$ | 1 ml |

## Description

2.5×STR Master Mixture is a premix system suitable for various types of multiple PCR at a concentration of 2.5×,Contains components such as DNA polymerase, PCR Buffer, dNTPs, $Mg^{2+}$, and stabilizers and enhancers, making the operation simple and fast.

The DNA polymerase contained in STR Master Mixture is a genetically engineered recombinant enzyme with 5'→3' DNA polymerase activity and 5'→3' exonuclease activity; DNA polymerase is modified by a new type of antibody, which is an antibody modified hot starter enzyme. It can effectively reduce the nonspecific amplification generated by the nonspecific binding of primers and templates or primer dimers under normal temperature conditions. It also has excellent characteristics such as short activation time, strong amplification ability, high sensitivity, and good stability. The combination of a unique PCR buffer system and hot start enzymes significantly improves the amplification efficiency of PCR, with higher sensitivity and stronger inhibitor tolerance.

This product has a wide range of applications, not only for general and dye based real-time fluorescence quantitative PCR, but also for forensic multiple STR amplification reactions. It can be used in forensic analysis, paternity testing, scientific research, and other aspects of human genetic identification.

## Notes

1. Before use, please gently mix the product upside down after it has completely melted, and use after briefly centrifuging.
2. Avoid repeated freezing and thawing of this product, as repeated freezing and thawing may degrade product performance. This product can be stored at - 20°C for a long time.

## Protocol

The following protocol is an example of conventional the STR detection reaction system and reaction conditions. In practical operation, The actual protocol should be improved and optimized based on specific uses, templates, primer structures, target fragment sizes, and amplification effects.

1. PCR reaction system

1.1. PCR reaction system (The nucleic acid of extracted ):

| Reagent | 10μL | 25μL | Final Concentration |
|---|---|---|---|
| 2.5×STR Master Mixture | 4 μL | 10 μL | 1× |
| 5×Primer Mix[1)] | 2 μL | 5 μL | 1× |
| Template DNA[2)] | X μL | X μL | |
| $ddH_2O$ | Up to 10 μL | Up to 25 μL | |

1.2. PCR reaction system (The Blood Spots direct amplification ):

| Reagent | 10μL | 25μL | Final Concentration |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 2.5×STR Master Mixture | 4 μL | 10 μL | 1× |
| 5×Primer Mix | 2 μL | 5 μL | 1× |
| Blood Spots size | 1.0mm | 1.2mm | |
| $ddH_2O$ | Up to 10 μL | Up to 25 μL | |

Note:1)When designing primers, minimize the difference between Tm of each primer, and try to control the difference within 5℃. 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 to optimize the reaction system.In order to achieve the optimal amplification effect, it is recommended that the primer mixture be used after a brief centrifugation with vortex oscillation for 10 seconds before use.

2)Generally, the amount of DNA template is based on 0.1ng-1ng human genomic DNA, and the amount of template input can be adjusted based on the amplification effect to determine the optimal template usage.

3)During the operation process, human genome contamination should be avoided, and it is recommended to set up a group of negative controls (without DNA) during the experiment

2. PCR reaction program:

| Step | Temperature | Time | cycles |
|---|---|---|---|
| Predenaturation | 95℃ | 5 s-2 min | 1 |
| Denaturation | 95℃ | 5 s | 28-31[4)] |
| Annealing/Extend | 55-65℃[1)] | 90-150 s[2)] | |
| Final extension | 60℃ | 10-40 min[3)] | |

Note:1)A two-step PCR reaction procedure is recommended. If good experimental results cannot be obtained due to low Tm values of primers or large differences in Tm values between primers, a three-step PCR amplification method can be tried. The annealing temperature should be within the range of 55℃ to 65℃ as a setting reference (the annealing temperature is usually 5℃ lower than the Tm value), and the extension temperature should be set at 72℃.

2) When a good amplification effect is not achieved, the annealing and extension time can be appropriately extended to 120s to 150s.

3) When incomplete terminal addition of A occurs in PCR product detection,, the final extension time can be appropriately extended to 30-40 minutes.

4) The number of cycles can be set based on the downstream application of the amplification product. If the number of cycles is too small and the amplification amount is insufficient, the recommended number of cycles is 28-31 cycles.

5) Blood card direct expansion can increase the 72℃ cleavage step according to the actual amplification effect to improve the amplification efficiency.

6) When using the ABI 9700 thermal cycler, please perform amplification in MAX mode.

7) PCR products can be stored at 2-8℃ for short term storage or at - 20℃ for long-term storage.