

2.5×TFS Master Mixture

Catalog Number: T666123 (1 mL)

Storage condition: -20℃, try to avoid repeated freezing and thawing.

Products content

Component	1 mL
2.5×TFS Master Mixture	1 mL
ddH ₂ O	1 mL

Products Introduction

The 2.5×TFS Master Mixture is a premixed system for all types of multiplexed PCRs. It contains DNA polymerase, PCR Buffer, dNTPs, Mg²⁺, stabilizers and enhancers at a concentration of 2.5×, which makes it easy and fast to use.

The DNA polymerase contained in the 2.5×TFS Master Mixture is a genetically engineered recombinant enzyme with 5'→3' DNA polymerase activity and no 5'→3' exonuclease activity; it is a new antibody-modified hot-start enzyme that can effectively reduce non-specific amplification caused by non-specific binding of primer and template or primer dimerization at room temperature. The DNA polymerase is modified by a new type of antibody, which is an antibody-modified hot-start enzyme, and can effectively reduce the non-specific amplification caused by the non-specific binding of primers and templates or primer dimerization at room temperature, and at the same time, it has the excellent features of short activation time, strong amplification ability, high sensitivity, good stability, etc. The unique PCR buffer system and the hot-start enzyme are suitable for the PCR. The unique combination of PCR buffer system and hot starter enzyme significantly improves the PCR amplification efficiency, sensitivity and inhibitor tolerance.

The product has a wide range of applications, not only for general and dye-based real-time fluorescence PCR, but also for forensic multiple STR amplification reaction, which can be used in forensic analysis, parentage identification and scientific research and other human genetic identification.

caveat

1. Before use, please mix the product gently by turning it up and down after it is completely melted and centrifuged briefly.

2. Avoid repeated freezing and thawing of the product, which may degrade its performance.

This product can be stored at -20℃ for a long time.

Usage

The following examples are STR reaction systems and conditions, which should be improved and optimized according to the specific use, template, primer structure, target fragment size and amplification effect.

PCR reaction system

Extract the DNA amplification reaction system:

reagents	10 μ L system	25 μ L system	final concentration
2.5 $\times$ TFS Master Mixture	4 μ L	10 μ L	1 $\times$
5 $\times$ Primer Mix ¹⁾	2 μ L	5 μ L	1 $\times$
Template DNA ²⁾	X μ L	X μ L	
ddH ₂ O	Up to 10 μ L	Up to 25 μ L	

Blood Card Direct Expansion Reaction System:

reagents	10 μ L system	25 μ L system	final concentration
2.5 $\times$ TFS Master Mixture	4 μ L	10 μ L	1 $\times$
5 $\times$ Primer Mix	2 μ L	5 μ L	1 $\times$
Blood card sample size	1.0 mm	1.2 mm	
ddH ₂ O	Up to 10 μ L	Up to 25 μ L	

Attention:

1) When designing the primers, the difference between the T_m of each primer should be minimized, and the difference should be controlled within 5 $^{\circ}$ C as far as possible. If the amplification efficiency is not high, the concentration of primers can be increased; if non-specific amplification occurs, the concentration of primers can be decreased to optimize the reaction system. For optimal amplification, it is recommended that the primer mixture be vortexed for 10 s and centrifuged briefly before use.

(2) The amount of DNA template is usually 0.1 ng-1 ng of human genomic DNA as a reference, and the amount of template input can be adjusted according to the amplification effect to determine the optimal amount of template to use.

3) Human genome contamination should be avoided during the operation, and a set of negative control (no DNA) is recommended for the experiment.

2. PCR reaction conditions

move	temp	timing	circulate
premutability	95 $^{\circ}$ C	5 s-2 min	1
denaturation	95 $^{\circ}$ C	5 s	28-31 ⁴⁾
Annealing, Extension	55-65 $^{\circ}$ C ¹⁾	90-150 s ²⁾	28-31 ⁴⁾
ultimate extension	60 $^{\circ}$ C	10-40 min ³⁾	

Attention:

- 1) Two-step PCR reaction program is recommended. If you can not get good results due to low T_m value of the primers or large difference in T_m value between primers, you can try to use three-step PCR amplification, the annealing temperature should be set in the range of 55°C-65°C as a reference (the annealing temperature is usually 5°C lower than the T_m value), and the extension temperature should be set at 72°C.
- (2) When good amplification results are not obtained, the annealing and extension time can be appropriately prolonged to 120 s-150 s. The annealing and extension time can be extended to 120 s-150 s.
- (3) When the PCR product detection appears to be incomplete plus A, the final extension time can be appropriately extended to 30-40 min.
- 4) The number of cycles can be set according to the downstream application of the amplified product, if the number of cycles is too small, the amplification is insufficient, the recommended number of cycles is 28-31 cycles.
- 5) Blood card direct amplification can be based on the actual amplification effect to increase the 72 °C lysis step to improve the amplification efficiency.
- 6) When using the ABI 9700 Thermal Cycler, perform amplification in MAX mode.
- 7) PCR products can be stored at 2-8°C for short-term storage or at -20°C for long-term storage.

reagents	50 μ l system	25 μ l system	20 μ l system	final concentration
2×HyperProbe Mixture	25 μ l	12.5 μ l	10 μ l	1×
Forward Primer, 10 μ M	1 μ l	0.5 μ l	0.4 μ l	0.2 μ M1)
Reverse Primer, 10 μ M	1 μ l	0.5 μ l	0.4 μ l	0.2 μ M1)
Probe2)				
Template DNA3)	X μ l	X μ l	X μ l	
ddH2O	up to 50 μ l	up to 25 μ l	up to 20 μ l	

Attention:

- (1) Usually, a primer concentration of 0.2 M can give better results, and 0.1-1.0 μ M can be used as a reference for setting the range. If the amplification efficiency is not high, the concentration of primer can be increased; if non-specific reaction occurs, the concentration of primer can be decreased to optimize the reaction system.
- (2) The final concentration of the probe used is related to the fluorescence quantitative PCR instrument used, the type of probe, and the type of fluorescent labeling substance, please refer to the manual of the instrument or the specific requirements for the use of each fluorescent probe to adjust the concentration.

3) Usually the amount of DNA template is 10-100 ng of genomic DNA or 1-10 ng of cDNA as a reference, as templates of different species

The number of copies of the target gene contained in them varies, and a gradient dilution of the template can be performed to determine the optimal amount of template to use.

PCR reaction conditions

move	temp	timing	
premutability	95°C	30s1)	
denaturation	95°C	10 s	40-45 cycles
Annealing/Extension	58°C	20 s2)	40-45 cycles

ention:

(1) The enzyme used in this product is activated by pre-denaturation at 95°C for 30 s. Most of the templates can be deconvoluted well under this condition. Under this condition, most of the templates can be well unchained. For templates with high GC content and complex secondary structure, the pre-denaturation time can be extended to 1 minute, so that the starting template can be fully unchained, and if the high temperature treatment time is too long, it will affect the activity of the enzyme; for simple templates, pre-denaturation can also be used for 20 s, and the optimal pre-denaturation time can be determined according to the template situation.

(2) It is recommended to use two-step PCR program, the annealing temperature should be 58-64°C as the reference range, and the annealing temperature can be increased in case of non-specific reaction. If you can't get good results due to the use of primers with low T_m value, you can try three-step PCR amplification, and the annealing temperature should be set in the range of 56°C-64°C as a reference. The annealing and extension times for several common instruments are shown in the following table: 20 s for Roche, BioRad, Agilent, Hongshi, Dongshenglong, etc. 30 s for ABI 7000/7300/7500. The annealing/extension times can be set according to the different types of instruments and templates, please follow the instructions of the instruments. The annealing/extension time can be set according to different models of instruments and templates, please follow the instruction manual of the instrument.