Tinzyme Co., Limited

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2×pfu PCR Super MasterMix

Product Number: PCM11

Shipping and Storage

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

Components

Component	PCM11	PCM11
2×pfu PCR Super MasterMix	1mL	5×1mL
ddH_2O	1mL	5×1mL

Description

This product is a premixed system composed of Pfu DNA Polymerase, Mg^{2+} , dNTPs, and PCR stabilizers and enhancers at a concentration of 2× . Pfu DNA Polymerase is a fast, high-efficiency, high-fidelity DNA polymerase with 5'-3' DNA polymerase activity and 3'-5' exonuclease activity. This polymerase is modified from other high-fidelity enzymes, has strong amplification ability, rapid amplification speed (4-6 kb/min), and high fidelity. This polymerase overcomes some defects of Pfu polymerase such as the poor amplification ability, low yield and amplification rate, which greatly shortens the reaction time.

The formulation of MasterMix makes the entire reaction system very stable, and suitable for the amplification of various fragment templates, minimizing human error and contamination. This product does not contain dyes, and an appropriate amount of sample loading buffer should be added for electrophoresis. The PCR product does not have an "A" base at the 3' end and can be directly used for blunt-end cloning. For T/A cloning, it is necessary to add "A" to the end of the PCR product.

Quality Control

No exogenous nuclease activity was detected; Can efficiently amplify various kinds of DNA templates; No apparent activity change after being stored at 2-8°C for one month.

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 template, primer structure and the size of the target.

1. PCR reaction system:

Regent	50μl reaction system	Final Conc.
2×pfu PCR Super MasterMix	25μl	1×
Forward Primer, 10 μM	2.5μl	0.5μM
Reverse Primer, 10 μM	2.5μl	0.5μM
Template DNA	适量	<250ng/50μl
ddH_2O	up to 50μl	

2. PCR reaction condition:

Step	Temperature	Time	Cycles
Pre denaturation	98°C	30s-30min	
Denaturation	98°C	5-10s	25-35
Annealing	45-72°C	10-30s	
Extend	72°C	4-6kb/min	
Final extension	72°C	5-10min	

Note:1) Denaturation: Simple templates are pre denatured at 98°C for 30 s-1 minutes. For more complex templates, the pre denaturation time can be extended to 3 minutes.

2)Annealing: In general experiments, the annealing temperature is 3-5℃ lower than the melting temperature Tm of the primer. If ideal amplification efficiency cannot be achieved, the annealing temperature should be changed gradient for optimization. When non-specific reactions occur, the annealing temperature should be appropriately increased. For primers with high Tm, two-step PCR can be used.

3)Extension: The extension time should be set based on the length of the amplified fragment and the complexity of the template. The amplification efficiency of this product is 4-6 kb/min, and can reach 6 kb/min for templates with low complexity.

4)Number of cycles: 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, or the number of cycles is too many, the probability of mismatch will increase, and the non-specific background is severe. Therefore, while ensuring product yield, the number of cycles should be minimized as much as possible.