Tinzyme Co., Limited

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2×pfu PCR Super MasterMix, with blue dye

Product Number: PCM11B

Shipping and Storage

-20 ℃. For frequent use, it can be stored at 2-8℃.

Components

Component	PCM11B 1mL	PCM11B 5mL
2×pfu PCR Super MasterMix, with blue dye	1mL	5×1mL
ddH_2O	1mL	5×1mL

Note: 2×pfu PCR Super MasterMix, with blue dyecontains Pfu DNA Polymerase, 3mM $MgCl_2$ and 400μM each dNTP。

Description

This product is a premixed system composed of Pfu DNA Polymerase, Mg^{2+}, dNTPs, PCR stabilizers and enhancers, with a concentration of 2 ×。 Pfu DNA Polymerase has 5 '-3' DNA polymerase activity and 3 '-5' exonuclease activity, thus possessing error correction ability during DNA amplification.Compared with Taq DNA Polymerase, it has high fidelity (6-8 times that of Taq enzyme) and better thermal stability. The pre prepared PCR mixture makes the operation simpler and faster, minimizing human error and pollution. The original MasterMix formula makes the entire reaction system very stable and has good repeatability. This product has been added with a dye (blue) and can be directly subjected to electrophoresis detection after the reaction is completed. The Pfu DNA polymerase contained in this product has the characteristics of low mismatch rate and high temperature resistance, making it suitable for gene cloning, gene site-specific mutations, SNP, and terminal complement reactions.

Quality Control

After testing, there was no exogenous nuclease activity; PCR method for detecting non host residual DNA; it can effectively amplify single copy genes from multiple genomes; Storage at 2-8 ℃ for three months showed no significant change in activity.

Protocol

The following example is a PCR reaction system and reaction conditions for amplifying a 1kb fragment using human genomic DNA as a template. In actual operation, corresponding improvement and optimization should be carried out according to the different template, primer structure and target fragment size.

1. PCR reaction system

Reagent	50μL reaction system	Final Concentration
2×pfu PCR Super MasterMix, with blue dye	25μl	1×
Forward Primer，10μM	2μl	0.4μM
Reverse Primer，10μM	2μl	0.4μM
Template DNA	< 0.5μl	<0.5μg/50μl
ddH_2O	Up to 50μl	

Note:When amplifying with Pfu enzyme, the purity of the primer is required to be high, and the primer length is greater than 18 bases. The primer concentration should be set at the final concentration of 0.1-1.0μM as the setting range reference for enclosure.In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific reactions occur, the concentration of primers can be reduced to optimize the reaction system.

2. PCR reaction condition

Step	Temperature	Time
Pre denaturation	94°C	2min

Denaturation	94°C	30s	25-35cycles
Annealing	55-65°C	30s	
Extend	72°C	60s	
Final extension	72°C	5min	

Note:1)The thermal stability of Pfu enzyme is better than that of Taq enzyme. For templates with high GC content, the denaturation temperature can be increased to 98℃ without affecting the activity of Pfu enzyme.

2) 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.

3) Pfu enzyme has 3 '-5' exonuclease activity, so the extension speed of Pfu enzyme amplification is much lower than that of Taq enzyme. The extension time is set according to the size of the amplified fragment, and the amplification extension rate of this product is 1kb/min.

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, 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.

5) This product has 3 '-5' exonuclease activity, and the PCR product is flat ended and cannot be directly used for T/A cloning. If T/A cloning is required, "A" needs to be added to its end or cloned using a flat ended vector.