Tinzyme Co., Limited

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2×Super Kfu MasterMix

Product Number: PCM313

Shipping and Storage

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

Components

Component	PCM313S 1ml	PCM313M 5ml
2×Super Kfu MasterMix	1 ml	5×1 ml
ddH_2O	1 ml	5×1 ml

Introduction

This product is a premixed system composed of Kfu DNA Polymerase, Mg^{2+}, dNTPs, PCR stabilizers and enhancers, with a concentration of 2×。Kfu DNA Polymerase is a fast and highly efficient high-fidelity DNA polymerase with 5'-3' DNA polymerase activity and 3'-5' exonuclease activity. It has advantages such as strong amplification ability, high fidelity, and strong specificity.2× Mix has added unique amplification enhancing factors and elongation factors, and the unique formula makes the entire reaction system very stable and easy to operate, suitable for amplification of various fragments and templates.This product is suitable for gene cloning, second-generation library building amplification, gene directed mutation, SNP and other amplification experiments.

Quality Control

After testing, there is no exogenous nuclease activity, which can effectively amplify various templates; Stored at 2-8℃ for one month, there was no significant change in activity.

Protocol

The following examples are the conventional PCR reaction system and reaction conditions. In practical operation, corresponding improvements and optimizations should be made based on different templates, primer structures, and target fragment sizes.

1. PCR reaction system

All operations should be carried out on ice. After each group is decomposed and frozen, please mix thoroughly. After use, please put it back at -20℃ for storage in a timely manner.

Reagent	50μL reaction system	Final Concentration
2×Super Kfu MasterMix	25μl	1×
Forward Primer, 10 μM	2μl	0.4μM
Reverse Primer, 10 μM	2μl	0.4μM
Template DNA appropriate amount	appropriate amount	<500ng/50μl
ddH_2O	up to 50μl	

2. PCR reaction condition

Step	Temperature	Time	
Predenaturation	98°C	30 s-3 min	
Denaturation	98°C	10-30 s	25-35cycles
Annealing	Based on primer Tm	15-30 s	
Extend	72°C	4-6 kb/min	
Final extension	72°C	5 min	

Note:1)Priority should be given to using the three-step amplification method. If the three-step method cannot amplify the target product or the Tm value of the primer is greater than 68°C, please try the two-step method.

2)Denaturation: Pre denaturation of simple templates at 98°C for 30 seconds to 1 minute. For complex templates, the pre denaturation time can be extended to 3 minutes.

3)Annealing: In general experiments, the annealing temperature is 3-5°C lower than the Tm value of the primer. If the ideal amplification efficiency cannot be achieved, the annealing temperature should be gradient changed for optimization; When non-specific reactions occur, appropriately increase the annealing temperature

4)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 2-4 kb/min is recommended for long fragments and high complexity templates

5)Number of cycles: The number of cycles can be set based on the downstream application of the amplified product. If the number of cycles is too small, the amplification amount is insufficient, and the number of cycles is too many, the probability of mismatch will increase. Therefore, while ensuring product yield, the number of cycles should be minimized as much as possible.