Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

2×Super Kfu MasterMix (Dye)

Product Number: PCM313B

Shipping and Storage

-20°C.

Components

Component	PCM313B 1ml	PCM313B 5ml
2×Super Kfu MasterMix (Dye)	1ml	5×1ml
ddH_2O	1ml	5×1ml

Description

This product is a premixed system composed of 2×Super Kfu MasterMix (Dye), 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. This product has been added with a dye (blue), and after the reaction, it can be directly detected by agarose electrophoresis, making the operation convenient and simple.

Quality Control

After testing, there is no exogenous nuclease activity and no residual host DNA, which can effectively amplify various templates.

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

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.

Component	50μL reaction system	Final Concentration
2×Super Kfu MasterMix (Dye)	25μL	1×
Forward Primer，10μM	2μL	0.4μM
Reverse Primer，10μM	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	30s-3min	
Denaturation	98°C	10-30s	25-35cycles
Annealing	Based on primer Tm	15-30s	
Extend	72°C	4-6kb/min	

Final extension	72°C	5min

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: Predenaturation of simple templates at 98°C for 30 seconds to 1 minute. For complex templates, the predenaturation 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, increase the annealing temperature appropriately.

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 4-6kb/min, and 2-4kb/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.