Tel : 86-10-56953015
Email : info@cwbio.com

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2×Super Kfx MasterMix

Cat. No. : CW3313S (1 mL)
CW3313M (5 mL)

Storage Condition: -20 ℃. Store at 2-8 ℃ for frequent use.

Components

Component	CW3313S 1 mL	CW3313M 5 mL
2×Super Kfx MasterMix	1 μL	5×1 mL
ddH$_2$O	1 μL	5×1 mL

Introduction

This product is a premix composed of Super Kfx DNA Polymerase, Mg2+, dNTPs, PCR stabilizer and enhancer with a concentration of 2×. Super Kfx DNA Polymerase is a fast, high amplification efficiency and high-fidelity DNA polymerase, which has 5 '-3' DNA polymerase activity and 3 '-5' exonuclease activity, and has the advantages of strong amplification ability, high fidelity and strong specificity. The unique amplification enhancer and extension factor are added to 2×Mix, and the unique formula makes the whole reaction system very stable and easy to operate, which is suitable for the amplification of various fragments and templates. This product is suitable for amplification experiments such as gene cloning, NGS library amplification, gene site-specific mutation, SNP, etc.

Quality Control

No exogenous nuclease activity was detected. No significant change in activity was detected after being stored at 2-8 ℃ for one month.

Procedure

The following examples are the conventional PCR reaction system and reaction conditions, which should be improved and optimized according to different template, primer structure and target fragment size in actual operation.

1. PCR reaction system

All operations should be carried out on the ice. After thawing, mix the components thoroughly and put them back to -20 ℃ for storage.

Reagent	50 μL System	Final Concentration
2×Super Kfx MasterMix	25 μL	1×
Forward Primer, 10 μM	2 μL	0.4 μM
Reverse Primer, 10 μM	2 μL	0.4 μM
Template DNA	(appropriate amount)	<500 ng/50 μL
ddH_2O	to 50 μL	

2. PCR program

Procedure	Temperature	Time	
Predenaturation	98 ℃	30 s -3 min	
Denaturation	98 ℃	10-30 s	25-35 cycles
Annealing	According to primer Tm	15-30 s	
Extension	72 ℃	4-6 kb/min	
Final Extension	72 ℃	5 min	

Note:1) The three-step method is preferred for amplification. If the target product or primer Tm value is greater than 68 ℃ cannot be amplified by the three-step method, please try the two-step method.
2) Denaturation: The predenaturation of simple template should be set to 98 ℃, 30 s-1min. For complex template, the predenaturation time can be extended to 3 min.
3) Annealing: In general, the annealing temperature is 3-5 ℃ lower than the Tm value of the primer. If the ideal amplification efficiency cannot be obtained, the annealing temperature should be changed on a gradient to optimize: when non-specific reaction occurs, the annealing temperature should be appropriately increased.
4) Extension: The extension time should be determined according to the length of the amplified fragment and the complexity of the template. The amplification efficiency of this product is 4-6 kb/min. For long fragments and templates with high complexity, 2-4 kb/min is recommended.
5) Number of cycles: The number of cycles can be set according to the downstream application of the amplification product. If the number of cycles is too low, there is insufficient amplification. If the number of cycles is too high, the mismatch rate increases, leading to significant nonspecific background. Therefore, the number of cycles should be minimized on the premise of ensuring the yield of the product.