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Super Kfx DNA Polymerase

Cat. No. : CW3312S (100 U)
CW3312M (500 U)

Storage Condition: -20 ℃

Components

Component	CW3312S 100 U	CW3312M 500 U
Super Kfx DNA Polymerase, 2 U/μL	50 μL	250 μL
2xSuper Kfx Buffer	2x1.25 mL	7x1.8 mL
dNTP Mix，10 mM each	150 μL	750 μL

Introduction

Super Kfx DNA Polymerase is a rapid and efficient high fidelity DNA polymerase with 5'-3'DNA polymerase activity and 3'-5 'exonuclease activity. The enzyme is modified by other high-fidelity enzymes, adding unique extension factor and specific promotion factor, which greatly improves the amplification ability, overcomes the defects of ordinary PCR amplification ability, low yield and slow amplify speed, and shortens the reaction time. This product can be used for the amplification of common fragments, long fragments and other complex templates. The 3' ends of the amplified PCR product do not carry "A" base. If T/A cloning is needed, it is necessary to add" A" to the end of the PCR product for cloning. This product is suitable for gene cloning, NGS library preparation amplification, gene site-specific mutation, SNP and other amplification experiments.

Activity Definition

The amount of enzyme required to incorporate 10 nmoL of deoxyribofirst acid into an acidic insoluble substance within 30 minutes at 74 ℃ is defined as 1 unit of activity (U).

Quality Control

After column purification for several times, the purity was more than 98% according to SDS-PAGE test. No exogenous nuclease activity was detected. No significant change in activity was detected after being stored at room temperature for one month.

Protocol

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
2xSuper Kfx Buffer	25 μL	1×
dNTP Mix，10 mM each	1.5-2.5 μL	300-500 μM each
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
Super Kfx DNA Polymerase	0.5-0.75 μL	1-1.5 U/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.