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Version: 09/2022

Super Pfx DNA Polymerase

Cat. No. : CW2848S (100 U)
CW2848M (500 U)

Storage Condition : -20°C

Components

Component	CW2848S 100 U	CW2848M 500 U
Super Pfx DNA Polymerase，2U/μL	50 μL	250 μL
2×Super Pfx Buffer	2×1.25 mL	7×1.8 mL
dNTP Mix，10mM each	150 μL	750 μL

Principle

Super Pfx DNA Polymerase is a fast and high-fidelity DNA Polymerase with high amplification efficiency. The Polymerase possesses 5 '-3' DNA Polymerase activity and 3 '-5' exonuclease activity. The enzyme was modified by other high-fidelity enzymes with strong amplification ability, fast amplification speed and high fidelity, which overcame the defects of ordinary Pfu enzyme such as poor amplification ability, low yield and slow amplification speed, and greatly shortened the reaction time. This product can be used for long fragment amplification and the expansion of other complex templates. The 3 'end of the amplified PCR product does not contain "A" base, and can be directly cloned in the flat terminal vector. If T/A cloning is needed, "A" should be added to the end of the PCR product for cloning. This product is suitable for gene cloning, gene point mutation, SNP amplification experiments.

Activity Definition

Absolute ethanol, 10 mM PBS (PH7.4)

Quality Control

After multiple column purification, the purity was more than 98% by SDS-PAGE. No exogenous nuclease activity was detected. After one month at room temperature, there was no obvious change of activity.

Procedure

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

1. PCR Reaction System

All operations should be carried out on ice. After thawing, mix the components thoroughly. After use, please put them back to -20°C in time.

Reagent	50 μL Reaction System	Final Conc.
2×Super Pfx 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)	Appropriate	<500 ng/50 μL
Super Pfx DNA Polymerase	0.5-0.75 μL	1-1.5 U/50 μL
ddH_2O	up to 50 μL	

2. PCR Reaction Program

Step	Temperature	Time	Cycles
Initialization	98°C	30 sec-3 min	1
Denaturation	98°C	10-30sec	25-35
Annealing	According to Tm	15-30 sec	
Extension	72°C	3-5kb /min	
Final Extension	72°C	5 min	1

Note:

1) The three-step amplification method is preferred. The three-step method cannot amplify the target product or primer with Tm value greater than 68°C, so please try the two-step method.

2) Denaturation: the pre-denaturation of simple templates is 98℃, 30s to 1min, and the pre-denaturation time can be extended to 3min for complex templates.

3) Annealing: in general experiments, 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 gradient for optimization; When non - specific reaction occurs, the annealing temperature should be increased appropriately.

4) Extension: The extension time should be set according to the length of the amplified fragment and the complexity of the template. The amplification efficiency of this product is 3-5 KB /min, and 2-4 KB /min is recommended for long fragments and templates with high complexity.

5) Cycle number: the cycle number can be set according to the downstream application of the amplification product. If the cycle number is too small, the expansion increment is insufficient, and the cycle number is too large, the mismatch probability will increase. Therefore, the cycle number should be reduced as far as possible on the premise of ensuring the yield of the product.as possible yet ensuring the yield of the product.