Super Pfx DNA Polymerase

Catalog Number:

S665588 (100 U)
S665588 (500 U)

Storage condition: -20℃

Products content

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

Products Introduction

Super Pfx DNA Polymerase is a fast, high-fidelity DNA polymerase with high amplification efficiency, which has 5′-3′ DNA polymerase activity and 3′-5′ exonuclease activity. The enzyme has been modified from other high-fidelity enzymes, with strong amplification ability, fast amplification speed and high fidelity, overcoming the defects of poor amplification ability, low yield and slow amplification speed of ordinary Pfu enzyme, and greatly shortening the reaction time. This product can be used for long fragment amplification and other complex templates, the 3′ end of the amplified PCR product does not have "A" base, it can be directly cloned in the flat end vector, if you need to carry out T/A cloning, it is necessary to add "A" at the end of the PCR product for cloning. If T/A cloning is needed, it is necessary to add "A" at the end of the PCR product for cloning. This product is suitable for gene cloning, targeted mutation and SNP amplification experiments.

Active Definition

The amount of enzyme required to incorporate 10 nmoL of deoxyribonucleotide into an acid-insoluble substance is defined as 1 active unit (U) at 74°C for 30 min.

quality control

After several column purifications, the purity of the product was greater than 98% by SDS-PAGE; no exogenous nuclease activity was detected; and the product was stored at room temperature for one month without any obvious activity change.

Usage

The following are examples of conventional PCR reaction systems and conditions, which should be improved and optimized according to the template, primer structure and fragment size.

1. PCR Reaction System All operations should be carried out on ice, and the components should be mixed well after thawing and stored at -20℃ after use.

reagents	50 μL reaction system	final concentration
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

TemplateDNA, moderate amount	appropriate amount	<500 ng/50 μL
Super Pfx DNA Polymerase	0.5-0.75 μL	1-1.5 U/50 μL
ddH2 O	up to 50 μL	

2. PCR reaction conditions

move	temp	timing	
premutability	98°C	30 s-3 min	
denaturation	98°C	10-30 s	25-35 cycles
annealing (metallurgy)	According to the primer Tm	15-30 s	25-35 cycles
reach	72°C	3-5 kb/min	25-35 cycles
ultimate extension	72°C	5 min	

take note of

1) Priority is given to three-step amplification; if the three-step method fails to amplify the target product or if the primer Tm value is greater than 68° C, try the two-step method.

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

3) Annealing: In general, the annealing temperature is 3-5℃ lower than the Tm value of the primers. If the desired amplification efficiency cannot be obtained, the annealing temperature should be changed in a gradient to optimize the results; if a 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 fragments and the complexity of the template, the amplification efficiency of this product is 3-5 kb/min, for long fragments and templates with high complexity it is recommended that 2-4kb/min.

5) Cycling times: the number of cycles can be set according to the downstream application of the amplification product. If the number of cycles is too small, the amplification amount will be insufficient, and if the number of cycles is too large, the chance of mismatch will be increased, so the number of cycles should be minimized under the premise of guaranteeing the yield of the product.