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Super Pfx DNA Polymerase

Catalog Number: CW2848S (100 U); CW2848M (500 U)

Storage Condition: -20°C.

Kit Components:

Component	CW2848S (100 U)	CW2848M (500 U)
Super Pfx DNA Polymerase, 2 U/ul	50 ul	250 ul
5x Super Pfx HF Buffer	1.5 ml	4x1.8 ml
5x Super Pfx GC Buffer	1.5 ml	4x1.8 ml

Product Introduction:

Super Pfx DNA Polymerase is a fast, high-efficiency, high-fidelity DNA polymerase with 5'-3' DNA polymerase activity and 3'-5' exonuclease activity. This polymerase is modified from other high-fidelity enzymes, has strong amplification ability, rapid amplification speed (4-6 kb/min), and high fidelity. This polymerase overcomes some defects of Pfu polymerase such as the poor amplification ability, low yield and amplification rate, which greatly shortens the reaction time.

This product can be used for the amplification of long fragments and other various complex templates. The PCR product does not have an "A" base at the 3' end and can be directly used for blunt-end cloning. For T/A cloning, it is necessary to add "A" to the end of the PCR product.

This product has the characteristics of rapid extension, high amplification efficiency and high fidelity. It is suitable for experiments such as gene cloning, site-directed mutagenesis, and SNP amplification.

Unit Definition:

The amount of enzyme required to incorporate 10 nmol deoxynucleotide into the acidic insoluble material within 30 minutes at 74°C is defined as 1 activity unit (U).

Quality Control:

After multiple column purifications, the purity was >98% by SDS-PAGE; No exogenous nuclease activity was detected; No apparent activity change after being stored at room temperature for one month.

Protocol:

The following protocol is an example of conventional PCR reaction system and condition. The actual protocol should be improved and optimized based on the template, primer structure and the size of the target.

1. PCR reaction system:

Reagent	50 ul	Final Conc.
5xSuper Pfx HF Buffer	10 ul	1x
dNTP Mix, 10 mM each	1 ul	200 uM each
Forward Primer, 10 uM	2.5 ul	0.5 uM
Reverse Primer, 10 uM	2.5 ul	0.5 uM
DNA template	X ul	< 250 ng/50 ul
Super Pfx DNA Polymerase	0.5 ul	1 U/50 ul
ddH_2O	Up to 50 ul	

Note:

1) The 5x Super Pfx HF Buffer and 5x Super Pfx GC Buffer contain 7.5 mM Mg^{2+};

2) It is recommended to use 5x Super Pfx GC Buffer for complex templates and templates with high GC content.

2. PCR reaction program:

Step	Temperature	Time	
Initialization	98°C	30 s-3 mins	
Denaturation	98°C	5-10 s	25-35 cycles
Annealing	45-72°C	10-30 s	
Elongation	72°C	2-4 kb/min	
Final elongation	72°C	5-10 mins	

Note:

1) Denaturation: For simple DNA templates, the pre-denaturation temperature is 98°C and the pre-denaturation time is 30 s to 1 minute. For more complicated templates, the pre-denaturation time can be extended to 3 minutes.

2) Annealing: the annealing temperature should be the 3-5°C lower than the Tm of primer. If the ideal amplification efficiency cannot be obtained, the annealing temperature should be changed in a gradient to optimize. When non-specific reactions occur, the annealing temperature should be appropriately

increased. Two-step PCR can be used for primers with high Tm.

3) Elongation: The extension time should be set according to the length of the amplified fragment and the complexity of the template. The amplification efficiency of the Super Pfx DNA Polymerase is 4-6 kb/min. For simple templates, the rate can be 6 kb/min.
4) Cycles: The number of cycles can be set based on the downstream applications of the PCR product. If the number is too low, the amount of PCR product is insufficient; if the number is high, the probability of mismatch and the non-specific background are increased. Therefore, the number of cycles should be reduced as much as possible yet ensuring the yield of the product.