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2×Super Pfx MasterMix

Catalog Number: CW2965S (1 ml)
CW2965S (5 ml)

Storage Condition: -20°C; For frequent uses, store at 2-8°C.

Kit Components:

Component	CW2965S (1 ml)	CW2965M (5 ml)
2x Super Pfx MasterMix	1 ml	5x1 ml
ddH_2O	1 ml	5x1 ml

Product Introduction:

This product is a premixed system composed of Super Pfx DNA Polymerase, Mg^{2+}, dNTPs, and PCR stabilizers and enhancers at a concentration of 2×. 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.

The formulation of MasterMix makes the entire reaction system very stable, and suitable for the amplification of various fragment templates, minimizing human error and contamination. This product does not contain dyes, and an appropriate amount of sample loading buffer should be added for electrophoresis.

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.

Quality Control:

No exogenous nuclease activity was detected; Can efficiently amplify various kinds of DNA templates; No apparent activity change after being stored at 2-8°C 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.
2xSuper Pfx MasterMix	25 ul	1x
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
ddH_2O	Up to 50 ul	

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.