2. PCR reaction condition

Procedure	Temperature	Time	
Pre-denaturation	95 ℃	5-60 s	1
Denaturation	95 ℃	5-15 s	35-45 cycles
Annealing/Extension	55-65 ℃	30 s	

Note:1) The hot-start enzyme used in this product needs to be incubated at 95 ℃ for at least 5 s to activate the enzyme.

2) In general experiments, the annealing temperature is 5 ℃ lower than the melting temperature (Tm) of the amplified primer. The annealing temperature can be appropriately reduced when the ideal amplification efficiency cannot be obtained.

3) The extension time should be set according to the size of the amplified fragment.

4) 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.

Version: 04/2024

SuperFastStar DNA Polymerase (exo-)

Cat. No. : CW3305S (500 U)

Storage Condition: -20±5 ℃.

Components

Component	CW3305S 500 U
SuperFastStar DNA Polymerase (exo-) (5 U/μL)	100 μL
10×PCR Buffer	1.8 mL

Note: The 10×PCR Buffer in this product contains 15mM Mg^{2+}.

Introduction

SuperFastStar DNA Polymerase (exo-) is a hybrid of anti-Taq enzyme monoclonal antibody and Taq DNA Polymerase for Hot Start PCR. When using Taq enzyme antibody for PCR amplification, the combination of Taq enzyme antibody and Taq enzyme inhibits the activity of DNA polymerase before high temperature denaturation, which could effectively inhibit the non-specific annealing of primer and the non-specific amplification caused by primer dimer at low temperature. The Taq enzyme antibody is denatured in the initial DNA denaturation step of PCR reaction, and the polymerase activity is restored to achieve the hot start effect. The use of this product does not require special inactivation of Taq enzyme antibodies, and can be used under conventional PCR reaction conditions.

SuperFastStar DNA Polymerase (exo-) has 5'-3' DNA polymerase activity, but no 5'-3' exonuclease activity or 3'-5' exonuclease activity. Enzyme extension rate is 2 kb/min, and can amplify fragments up to 5 kb. The amplified PCR product has an " A " base attached to the 3' end and can therefore be used directly for T/A cloning. No polymerase activity is released at 55 ℃ and below, and DNA polymerase activity can be restored by heating at 95 ℃ for 5 s. This product has the characteristics of fast extension speed and high amplification efficiency, and is mainly suitable for DNA amplification with PCR, DNA sequencing and other experiments.

Notes

1. SuperFastStar DNA Polymerase (exo-) should be used after being removed from -20 ℃ and centrifuged immediately. The product can be operated at normal temperature. If the experimental temperature is higher than 25 ℃ or the experimental time is too long, please place the enzyme on ice. SuperFastStar DNA Polymerase (exo-) can be stored at -20 ℃ for at least 2 years.
2. The PCR Buffer can be stored at -20±5 ℃ for at least 2 years, and at 2-8 ℃ for at least 1 month if used frequently.
3. Do not freeze-thaw frequently (recommended freeze-thaw times ≤10).
4. This product is recommended to be used in small portions.

Quality Control

1. Protein purity: The purity is close to 99% by HPLC.
2. Exonuclease residue detection: 10 U of proenzyme and 0.6 μg λ-Hind III were incubated at 37 ℃ for 16 h, and the DNA electrophoretic bands did not change.
3. Endonuclease residue detection: 10 U of proenzyme and 0.6 μg Supercoiled pBR322 DNA were incubated at 37 ℃ for 4 h, and the electrophoresis bands of DNA did not change.
4. RNase residue detection: 10 U proenzyme and 1 μg HeLa cell total RNA were incubated at 37 ℃ for 1 h, and the electrophoretic bands of RNA did not change.

Protocol

The following example uses human genomic DNA as a template to amplify PCR reaction conditions and reaction systems of 1 kb fragments, and should be improved and optimized according to the template, primer structure and target fragment size in practice.

1. PCR reaction system

Reagent	50 μL System	Final Concentration
10×PCR Buffer	5 μL	1×
dNTP Mix,10mM each	1 μL	200 μM each
Forward Primer，10 μM	1 μL	0.2 μM
Reverse Primer，10 μM	1 μL	0.2 μM
Template DNA	< 5 μL	< 0.5 μg/50 μL
SuperFastStar DNA Polymerase(exo-) (5 U/μL)	0.25-0.5 μL	1.25-2.5 U/50 μL
ddH_2O	to 50 μL	

Note: The system can be prepared at room temperature.