Version: 11/2023

2. PCR reaction procedure

Steps	Temperature	Time	Circulate
Pre-denaturation	95°C	5-60 s	1
Denaturation	95°C	5-15s	35-45 cycles
Annealing/Extension	55-65°C	30 s	

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

2) In general experiments, the annealing temperature is 5 °C 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 small, the expansion is insufficient. If the number of cycles is too many, the probability of mismatch will increase, and the non-specific background will be severe. Therefore, the number of cycles should be minimized under the premise of ensuring the yield of the product.

This product is for scientific research only, which shall not be used for clinical diagnosis or other purposes.

SuperFastStar DNA Polymerase (Glycerol-free)

Cat. No. : CW3336S (500 U)
CW3336L (50 kU)
Storage Condition: -20±5 °C.

Components

Component	CW3336S 500 U	CW3336L 50 kU
SuperFastStar DNA Polymerase (Glycerol-free)(5 U/μL)	100 μL	10×1 mL

Introduction

SuperFastStar DNA Polymerase (Glycerol-free) is a mixture of glycerol-free 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 (Glycerol-free) has 5′-3′ DNA polymerase activity and 5′-3′ exonuclease activity, and no 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. The blocking rate of polymerase activity at a temperature of 55 °C and below is more than 95%, and DNA polymerase activity can be restored by heating at 95 °C for 5 s. This product has the characteristics of fast extension speed and high amplification efficiency, and is mainly suitable for lyophilization of single enzyme, multiplex amplification and DNA sequencing.

Notes

1. After taking out this product from -20 °C, please place it at 4 °C or room temperature to melt. Do not melt with hands.
2. After taking out this product, centrifuge it instantaneously before use. This product can be operated in a room temperature environment. If the experimental environment temperature is higher than 25 °C, or the experimental time is too long, please put the enzyme on ice.
3. Avoid repeated freeze-thaw of this product, because it may reduce the performance of the product. The frequency of freeze-thaw should be less than 10 times. This product can be stored at -20±5 °C for a long time.
4. This product is recommended for use in small portions.

Quality Control

1. Protein purity: HPLC detection purity is close to 99%.
2. Exonuclease residue assay: 10 U of enzyme and 0.6 μg λ-Hind III were incubated at 37°C for 16 h, and the electrophoretic bands of DNA did not change.
3. Endonuclease residue assay: 10 U of enzyme and 0.6 μg Supercoiled pBR322 DNA were incubated at 37°C for 4 h, and the electrophoretic bands of DNA did not change.
4. RNase residue detection: 10 U of enzyme and 1 μg of total RNA from HeLa cells were incubated at 37 °C for 1 h, and the electrophoretic bands of DNA 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
PCR Buffer	x 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	<0.5 μL	<0.5 μg/50 μL
SuperFastStar DNA Polymerase (Glycerol-free) (5U/μL)	0.25-0.5 μL	1.25-2.5 U/50 μL
ddH_2O	to 50 μL	

Note: The reaction system can be prepared at room temperature.