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FastStar DNA Polymerase (exo-)

Product Number:PC16E

Shipping and Storage

-20±5°C.

Components

Component	PC16E 500U
FastStar DNA Polymerase (exo-)(5 U/μL)	100μL
10×PCR Buffer	1.8mL

Note: The 10×PCR buffer of this product contains 15mM magnesium ions.

Description

FastStar DNA Polymerase (exo-) is a mixture of anti Taq enzyme monoclonal antibodies and Taq DNA Polymerase, suitable for Hot Start PCR.When using Taq enzyme antibodies for PCR amplification, the binding of Taq enzyme antibodies to Taq enzyme inhibits DNA polymerase activity before high-temperature denaturation, which can effectively inhibit non-specific annealing of primers and non-specific amplification caused by primer dimers under low temperature conditions.Taq enzyme antibodies undergo denaturation in the initial DNA denaturation step of PCR reaction, and DNA polymerase activity is restored, achieving a hot start effect. This product does not require special inactivation of Taq enzyme antibodies and can be used under conventional PCR reaction conditions.

FastStar DNA Polymerase (exo-) has 5'-3' DNA polymerase activity, without 5'-3' exonase activity and 3'-5' exonase activity. The enzyme has an elongation rate of 2 kb/min and can amplify fragments up to 5 kb in length.The amplified PCR product has an"A" base attached to its 3 'end, making it suitable for direct T/A cloning. At temperatures of 55°C and below, there is no release of polymerase activity. Heating at 95°C for 5 seconds can restore DNA polymerase activity.This product has the characteristics of fast extension speed and high amplification efficiency, and is mainly suitable for experiments such as PCR amplification of DNA fragments and DNA sequence determination.

Notes

1. FastStar DNA Polymerase (exo-) is taken out from -20°C and centrifuged instantaneously before use. This product can be operated at room temperature. If the experimental environment temperature is above 25°C or the experimental time is too long, please place the enzyme on ice. FastStar DNA Polymerase (exo-) can be stored at -20°C for at least 2 years.
2. Store the PCR buffer at -20±5°C for at least 2 years. If frequently used, it can be stored at 2-8°C for at least 1 month.
3. FastStar DNA Polymerase (exo-) and PCR Buffer are strictly prohibited from repeated freeze-thaw cycles (recommended freeze-thaw cycles ≤ 10).
4. This product is recommended for small portion packaging.

Quality control

1. Protein purity: HPLC method detects purity approaching 99%.
2. Detection of residual exonuclease: 10U of proenzyme and 0.6μg λ- Hind III was incubated at 37°C for 16 hours without any changes in the electrophoresis bands of DNA
3. Endonuclease residue detection: 10U of proenzyme and 0.6μg Supercooled pBR322 DNA was incubated at 37°C for 4 hours, and the electrophoresis bands of the DNA did not change.
4. RNase residue detection: 10U of proenzyme and 1μg The total RNA of HeLa cells was incubated at 37°C for 1 hour, and the electrophoretic bands of the RNA did not change.

Protocol

The following example is a PCR reaction system and reaction conditions for amplifying a 1kb fragment using human genome DNA as a template. In practical operation, corresponding improvements and optimizations should be made based on the template, primer structure, and target fragment size.

1. PCR reaction system

Reagent	50μL	Final Conc.
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
FastStar DNA Polymerase (exo-)(5 U/μL)	0.25-0.5 μL	1.25-2.5U/50 μL
ddH_2O	Up to 50 μL	

Note: The reaction system can be configured at room temperature

2. PCR reaction program:

Step	Temperature	Time	cycles
Pre denaturation	95°C	5-60 s	1
Denaturation	95°C	5-15 s	35-45
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 seconds to activate the enzyme.

2)In general experiments, the annealing temperature is 5°C lower than the melting temperature Tm of the amplification primer, and when ideal amplification efficiency cannot be achieved, the annealing temperature should be appropriately reduced.

3)The extension time should be set based on the size of the amplified fragment.

4)The number of cycles can be set based on the downstream application of the amplification product. If the number of cycles is too small, the amplification amount is insufficient; If there are too many cycles, the probability of mismatch will increase, and the non-specific background will be severe. So, while ensuring product yield, the number of cycles should be minimized as much as possible.