FastPrime DNA Polymerase

Catalog number: F665641 (500 U)
F665641 (2500 U)

Storage condition: -20℃

Products content

Component	500 U	F665641
FastPrime DNA Polymerase, 5 U/μl	100 μl	5×100 μl
10×PCR Buffer	1.8 ml	5 x 1.8 ml

Note: The 10 x PCR Buffer for this product contains 15 mM magnesium ions.

Products Introduction

FastPrime DNA Polymerase is a mixture of anti-Taq enzyme monoclonal antibody and Taq DNA Polymerase with high amplification and fidelity properties for HOT Start PCR. When using FastPrime DNA Polymerase for PCR amplification, Taq enzyme antibody binds to Taq enzyme and inhibits DNA polymerase activity before denaturation at low temperature. When using FastPrime DNA Polymerase for PCR amplification, before denaturation at high temperature, Taqase antibody binds to Taqase to inhibit DNA polymerase activity, which can effectively inhibit the non-specific annealing of primers and non-specific amplification caused by primer dimer at low temperature, and the Taqase antibody denatures during the initial DNA denaturation step of the PCR reaction, and the activity of DNA polymerase is restored, thus achieving the effect of Hot Start. No special inactivation of the Taqase antibody is required for the use of this product, and it can be used under conventional PCR reaction conditions.

FastPrime DNA Polymerase has 5′→3′ DNA polymerase activity, 5′→3′ exonuclease and 3′→5′ exonuclease activity. Compared with Taq DNA Polymerase, FastPrime DNA Polymerase has high amplification efficiency and low mismatch rate, and can amplify DNA fragments efficiently. The PCR products amplified with this product have an "A" base at the 3′ end, which can be directly used for T/A cloning. This product is suitable for routine PCR reactions and gene cloning reactions that require high fidelity.

Definition of activity

The amount of enzyme required to dope 10 nmol of deoxyribonucleotide into acid-insoluble material was defined as 1 activity unit (U) at 74°C for 30 min, using activated salmon sperm DNA as template/primer.

quality control

After several column purification, its purity is more than 99% by SDS-PAGE; no exogenous nuclease activity is detected; no host residual DNA is detected by PCR method; it can effectively amplify single-copy genes in the human genome.

Usage

The following is an example of a PCR reaction system and reaction conditions for amplifying a 1 kb fragment of human genomic DNA as a template, which should be improved and optimized according to the template, primer structure and size of the target fragment in actual operation.

1. PCR reaction system

reagents	50μl reaction system	final concentration
10× PCR Buffer	5 μl	1×
dNTP Mix, 10 mM each	1 μl	200 μM each
Forward Primer, 10 μM	2 μl	0.4 μM
Reverse Primer, 10 μM	2 μl	0.4 μM
Template DNA	<0.5 μg	<0.5 μg/50 μl
FastPrime DNA Polymerase,5U/μl	0.25-0.5 μ	1.25-2.5 U/50 μl
ddH2O	up to 50 μl	

Note: Please use the final concentration of 0.1-1.0 μM as a reference for setting the range of primer concentration. If the amplification efficiency is not high, the primer concentration can be increased; if a non-specific reaction occurs, the primer concentration can be decreased to optimize the reaction system.

2.PCR reaction conditions

move	temp	timing	
premutability	95°C	2 min	
denaturation	95°C	30 s	25-35 cycles
annealing (metallurgy)	55-65°C	30 s	25-35 cycles
reach	72°C	30 s	25-35 cycles
ultimate extension	72°C	2 min	

Attention:

1）In general, the annealing temperature is 5°C lower than the melting temperature of the amplification primer, Tm. When the desired amplification efficiency cannot be obtained, the annealing temperature should be lowered appropriately; when non-specific reaction occurs, the annealing temperature should be increased, so as to optimize the reaction conditions.

2）The extension time should be set according to the size of the amplified fragment, and the amplification efficiency of this product is 2 kb/min.

3) The number of cycles can be set according to the downstream application of the amplified product. If the number of cycles is too low, the amount of amplification will be insufficient; if the number of cycles is too high, the chance of mismatch will increase and the non-specific background will be serious. Therefore, the number of cycles should be minimized under the premise of ensuring the product yield.