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Version: 01/2021

FastPrime DNA Polymerase

Cat. No. : CW2977S (500 U)
CW2977M (2500 U)

Shipping and Storage : -20°C

Components

Component	CW2977S 500 U	CW2977M 2500 U
FastPrime DNA Polymerase, 5 U/μL	100 μL	5×100 μL
10×PCR Buffer	1.8 mL	5×1.8 mL

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

Principle

FastPrime DNA Polymerase is a mixture of Taq monoclonal antibody and Taq DNA Polymerase with high amplification and fidelity. It is suitable for HOT Start PCR. When using FastPrime DNA Polymerase in PCR amplification, the Taq enzyme antibody inhibited the activity of DNA polymerase before denaturation at high temperature, which effectively inhibited the non-specific annealing of primers and the non-specific amplification caused by primer dimers at low temperatures. The Taq enzyme antibody was denatured in the initial DNA denaturation step of PCR reaction, and the DNA polymerase activity gradually restored to achieve the thermal activation effect. This product does not require special inactivation of Taq enzyme antibodies and can be used under conventional PCR reaction conditions.

FastPrime DNA Polymerase has 5 '→3' DNA polymerase activity, 5 '→3' exonuclease activity and 3 '→5' exonuclease activity. Compared with Taq DNA Polymerase, FastPrime DNA Polymerase has high amplification efficiency and low mismatch rate, which can efficiently amplify DNA fragments. This product can be used directly for T/A cloning because the 3' end of the amplified PCR product has an "A" base. This product is suitable for routine PCR reaction and gene cloning for high fidelity reaction.

Activity Definition

After column purification, the purity was more than 99% according to SDS-PAGE. No exogenous nuclease activity was detected. No host residual DNA was detected by PCR. Effectively amplify single-copy genes in the human genome.

Procedure

The following examples are the PCR reaction system and reaction conditions for the amplification of 1 kb fragments using human genome DNA as a template. In actual operation, corresponding improvements and optimization should be made according to different template, primer structure and target fragment size.

1. PCR Reaction System

Reagent	50 uL Reaction System	Final Conc.
10×PCR Buffer	5 uL	1×
dNTP Mix, 10 mM each	1 uL	200 uM each
Forward Primer, 10 uM	2 uL	0.4 uM
Reverse Primer, 10 uM	2 uL	0.4 uM
Template DNA	<0.5 ug	<0.5 ug/50 uL
FastPrime DNA Polymerase	0.25-0.5 uL	1.25-2.5 U/50 uL
ddH_2O	Up to 50 uL	

Note: Primer concentration please use final concentration 0.1-1.0 μM as reference for the set range. When the amplification efficiency is not high, the concentration of primers can be increased. When non-specific reaction occurs, the concentration of primers can be reduced to optimize the reaction system.

2. PCR Reation condition

Step	Temperature	Time	
Initial denaturation	94 °C	2 mins	
Denaturation	94 °C	30 s	25-35 cycles
Annealing	55-65 °C	30 s	
Extension	72 °C	30 s	
Final Extension	72 °C	2 mins	

Note:

1) In general experiments, the annealing temperature is 5°C lower than the melting temperature of the amplification primer Tm, and when the ideal amplification efficiency cannot be obtained, the annealing temperature should be reduced properly; Annealing temperature is raised to optimize reaction conditions when non-specific reactions occur.

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

3) The cycle number can be set according to the downstream application of the amplified product. If the number of cycles is too small, the increment of 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 reduced as far as possible under the premise of ensuring the yield of products.