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Taq DNA Polymerase

Cat. No. : CW0680S (500 U)
CW0680M (2500 U)
CW0680L (10000 U)

Shipping and Storage : -20°C.

Components

Component	CW0680S 500 U	CW0680M 2500 U	CW0680L 10000 U
Taq DNA Polymerase，5 U/μL	100 μL	5×100 μL	2×1 mL
10×PCR Buffer	1.8 mL	5×1.8 mL	8×5 mL

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

Introduction

The Taq DNA Polymerase is a purified recombinant enzyme expressed by E. coli. The gene is derived from Thermus Aquaticus polymerase. The molecular weight of the protein is 94 kDa, and it has 5 '→3' DNA polymerase activity and 5 '→3' exonuclease activity, but no 3 '→5' exonuclease activity. The elongation rate of the enzyme was 2 KB /min, and the length of 5 KB fragment could be amplified. The amplified PCR product has an "A" base attached to its 3 'end, so it can be directly used for T/A cloning. This product has the characteristics of fast extension with high speed and high amplification efficiency, mainly suitable for PCR amplification of DNA fragments, DNA sequence determination and other experiments.

Activity definition

Using activated salmon sperm DNA as template/primer, the amount of enzyme required to incorporate 10 nmol deoxynucleotides into acidic insoluble material was defined as 1 active unit (U) at 74°C for 30 minutes.

Quality control

The purity is grater than 99% after column purification by SDS-PAGE. No exogenous nuclease activity was detected. PCR was used to detect residual DNA without host. It can effectively amplify single copy gene in human genome. There was no obvious activity change after one month storage at room temperature.

Procedure

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

1. PCR reaction system

Reagent	50 μL 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
Taq DNA Polymerase，5 U/μL	0.25-0.5 μL	1.25-2.5 U/50 μL
ddH_2O	up to 50 μL	

Note: Primer concentration should take final concentration 0.1-1.0 μM as reference for setting range. When the amplification efficiency is not high, the primer concentration can be increased. When nonspecific reactions occur, the concentration of primers can be reduced to optimize the reaction system.

2. PCR reaction condition

Step	Temperature	Time	
Initialization	94°C	2 min	
Denaturation	94°C	30 s	25-35 cycles
Annealing	55-65°C	30 s	
Elongation	72°C	30 s	
Final elongation	72°C	2 min	

Note:

1) In general experiments, the annealing temperature is 5°C lower than the melting temperature Tm of the amplification primer, so the ideal amplification efficiency cannot be obtained, the annealing temperature should be appropriately reduced; When the nonspecific reaction occurs, the annealing temperature is increased to optimize the reaction conditions.

2) The extension time should be set according to the size of the amplified fragment. The amplification efficiency of the Taq DNA Polymerase of the product is 2 KB /min.

3) Cycle number can be set according to downstream application of amplified products. If the number of cycles is too small, the increment of expansion is insufficient; If the number of cycles is too high, the mismatch rate will increase, and the non-specific background will be serious. Therefore, the number of cycles should be reduced as far as possible under the premise of ensuring the product yield.