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Product Manual

Product Name	Pfu DNA Polymerase
Source	Recombinantly expressed in Escherichia coli
Catalog Number	CSB-DEM035
Physical Form	Liquid
Enzyme Activity	1 U/μL
Storage Conditions	-20 ±5°C
Storage Buffer	10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5% Tween 20, 0.5% NP-40, 50% Glycerol
Activity Definition	The amount of enzyme required to incorporate 10 nmol of deoxynucleotides into acid-insoluble material in 30 minutes at 72°C is defined as 1 unit (U).
Quality Control	Free of exonuclease and endonuclease contamination
Shelf Life	24 months

Product Description

Pfu DNA Polymerase is a highly heat-stable DNA polymerase derived from the thermophilic bacterium Pyrococcus furiosus and recombinantly expressed in Escherichia coli. It exhibits high amplification efficiency and fidelity. Due to its strong 3' - 5' exonuclease activity, it possesses higher fidelity compared to Taq DNA Polymerase, along with efficient amplification capabilities at a speed of up to 1 kb/10s. Pfu DNA Polymerase generates blunt-ended products in the amplification reaction.

Product components

Component NO.	Component Name	Specifications		
1	5×Pfu buffer	0.6mL	3mL	6mL
2	Pfu DNA Polymerase	100 U	500U	1000 U

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Usage of Product Components

Recommended Reaction System

Components	Addition amount
ddH2O	Up to 30 μL
5×Pfu buffer	6 μL
10 mM dNTPs	0.6 μL
Forward Primer (10 μM)	0.4-1 μL
Reverse Primer (10 μM)	0.4-1 μL
Pfu DNA Polymerase	1 μL
Template DNA	X μL

a. Primer Concentration:

Generally, a primer concentration of 0.2 μM in the reaction system yields good results. Adjust the primer concentration within the range of 0.1 μM to 1.0 μM if the reaction performance is poor.

b. Template Concentration:

Genomic DNA from animals and plants: 0.1-1 μg, E. coli genomic DNA: 10-100 ng, λDNA: 0.1-10 ng, plasmid DNA: 0.1-10 ng. If using undiluted cDNA, the volume should not exceed 1/10 of the total qPCR reaction volume.

c. Polymerase Concentration:

The enzyme amount can be adjusted between 0.25-1 μL. Increasing the enzyme amount generally improves the amplification yield but may decrease specificity.

d. Mg2+ and Additives:

The Mg2+ concentration in most PCR reaction systems should be within the range of 1.0-5.0 mM. However, for difficult amplification samples such as high GC content DNA, additives like DMSO or formamide may need to be added to the PCR reaction system.

Recommended PCR Reaction Program

Temperature	Time	Number of cycles
95°C	3 min	1
95°C	10 s	
(TM-5)°C	20 s	25-35
72°C	1 kb/min	
72°C	5 min	1

e. Annealing Temperature and Time:

The annealing temperature should be adjusted based on the primer's Tm value, generally set 3-5°C below the primer's Tm value. The recommended annealing time is 20 seconds, adjustable within the range of 10-30 seconds