molecular designs

PRODUCT INFORMATION

Catalog No.: D151

Product Name: ***Pfu* DNA Polymerase**

Size: 1000 units

Concentration: 5 units/µl

Storage: Store at-20°C.

Description: *Pfu* DNA Polymerase is a thermostable DNA polymerase from Pyrococcus furiosus. The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'->3' direction. *Pfu* DNA Polymerase also exhibits 3'->5' exonuclease activity, that enables the polymerase to correct nucleotide incorporation errors (proofreading). It has no 5'->3' exonuclease activity. This *Pfu* DNA polymerase is purified from an *E. coli* strain expressing a *Pfu* DNA Polymerase gene of Pyrococcus furiosus. It can be used for PCR experiments that require high-fidelity DNA synthesis.

Unit Definition: One unit incorporates 10nmoles of dNTPs into acid-insoluble material in 30 minutes at 72°C.

Storage Buffer: 5 units/µl in 50mM Tris-HCl (pH8.0), 100mM NaCl, 0.1mM EDTA, 1mM DTT, 50% glycerol, 0.5% TritonX-100, and 0.5% NP-40.

Reaction Buffer(10X): 200mM TrisHCl(pH 8.8), 100mM KCl, 160mM $(NH_4)_2SO_4$, 20mM $MgSO_4$, 1% Triton X-100, 1mg/ml nuclease-free bovine serum albumin (BSA).

Protocol:

1. Assembling of the PCR reactions as following:

Components	Volume : µl	Final Conc.		Components	Positive	Negative
10x Pfu reaction buffer	5µl	1x		10x Pfu reaction buffer	5µl	5µl
2.5mM dNTP mixture	4µl	200µM each		2.5mM dNTP mixture	4µl	4µl
Forward primer	1µl	0.1-1µM		Forward primer	1µl	1µl
Reverse primer	1µl	0.1-1µM		Reverse primer	1µl	1µl
Pfu DNA polymerase	variable	2.5-5U/50µl		Pfu DNA polymerase	0.2µl	0.2µl
Template DNA	variable	See note 1		Control DNA Template	1µl	---
Water (PCR--Grade)	variable	---		Water (PCR--Grade)	32.8µl	33.8µl
Total Volume	50µl	---		Total Volume	50µl	50µl

2. Mix and perform PCR using the following cycling program:

Step	Temperature	Duration	Cycles
Initial denaturation	95°C	3min	1
Denature	95°C	30sec	25-36
Anneal	50-68°C	30sec	
Extension	72°C	60sec/kb	
Final extension	72°C	10min	1
Storage	4°C	Hold	

Recommendations for Optimal Results

1. For more robust amplification, add additional *Pfu* DNA polymerase as needed in 0.5 µl increments.
2. Template DNA needed: Genomic: 50-250ng; Plasmid: 1pg-10ng; Viral DNA: 1pg-10ng.
3. For optimization of PCR results, adjust annealing temperature and Mg_2^+ as needed.

This product is for research use only.