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PFU DNA POLYMERASE FS-T-004

Description
Pfu DNA Polymerase is a highly thermostable DNA polymerase from the hyperthermophilic archaeum Pyrococcus furiosus. The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'-+3' direction. Pfu DNA Polymerase also possesses 3'-+5' exonuclease (proofreading) activity. Pfu DNA Polymerase exhibits the lowest error rate of any thermostable DNA polymerase studied, is even up to ten fold more accurate than normal Taq DNA polymerase. Consequently,
Pfu DNA Polymerase is useful for polymerization reactions requiring high-fidelity synthesis.

Description	FS-T-004
Pfu Polymerase (2.5U/ul)	1000 units
10X Pfu Buffer	1 vial

Storage Buffer
20mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Tween 20, and 0.5% Nonidet P40

10X Pfu Buffer
200mM Tris-HCl, 100mM KCl, 100mM $(NH_4)_2SO_4$, 1% Triton X-100, 1mg/ml BSA, 20mM $MgSO_4$, pH 8.8 (25°C).

Unit Definition
1 unit of the enzyme catalyzes the incorporation of 10 nanomoles of deoxyribonucleotides into a polynucleotide fraction in 30 min at 70°C.

Concentration: 2,5 u/ul

Quality control
Free of detectable, non-specific nucleases.

Storage Conditions
Store all components at -20ºC in a non-frost-free freezer.

Application
- High-fidelity PCR and primer-extension reactions
- Generation of PCR products for cloning and expression.
- PCR cloning and blunt-end amplification product generation
- RT-PCR for cDNA cloning and expression
- Site-directed mutagenesis
- Blunt-end PCR cloning

Recommended Protocol
General PCR reaction mixture for 50 ul Reaction :
On ice, prepare each of following master mixes, combine, and place in heated (to 94°C) thermal cycler:

In a sterile, nuclease-free microcentrifuge tube, combine the following components:

For 50 ul PCR Reaction	Volume	Final Conc.
Pfu DNA Polymerase	0.2-1.0 ul	0.5 - 2.5 U
10X Pfu Buffer	5 ul	1 X
dNTP mix (2.5 mM each)	4 ul	200 PM each
Template	<500 ng	<500 ng
Forward Primer	5 - 50 pmol	0.1-1 uM
Reverse Primer	5 - 50 pmol	0.1-1 uM
Distilled water	up to 50 ul	

Recommended PCR Cycling Conditions :

Step	Temp (°C)	Time (min)	Cycle
Initial Denaturation	95	1 - 5	1
Denature	95	0.5 -1	25 - 40
Anneal	50-65	0.5	
Extend	72	0.5 - 1	
Final Extension	72	5	1

IMPORTANT: Annealing temperature should be 2-6ºC lower than the primer melting temperature. Elongation time should be -1 min/1 kb.

Notice :
Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

Quality Control Assay

Contamination Assay
Pfu DNA Polymerase was passed from quality control assay for contamination of bacterial host DNA using sequence-specific primer set from host bacterial genomic DNA.

Functional assay
Pfu DNA Polymerase was functionally tested for PCR amplifications using the various size from human genomic DNA.

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36 Terry Drive
Trevose, PA 19048 - USA