BIOMAN SCIENTIFIC CO,LTD

pfu DNA Polymerase

Cat No: PF500 **Sise:** 500u 5u/ul

Description

Pfu DNA polymerase is a thermostable enzyme isolated from Pyrococcus furiousus. The enzyme replicates DNA at 75°C, catalyzing the polymerization of nucleotides into duplex DNA in the 5'-3' direction.

Pfu DNA polymerase possesses 3'-5' exonuclease (proofreading) activity. Base misincorporation is rapidly excised by the proofreading activity of the polymerase. Pfu DNA polymerase is recommended for PCR and primer extension reactions that require high-fidelity. The fragments of Pfu DNA polymerase generated are blunt-ended.

Error rate : 2×10^{-6}

Unit description: One unit of Pfu DNA Polymerase incorporates 10 nmol of dNTP into acid-insoluble material in 30 min at 74°C

Storage buffer : 50% glycerol (v/v), 20 mM Tris-HCl pH 8.7 at -20°C, 100 mM KCl, 0.1 mM EDTA.

Reaction Buffer (10x) with MgSO4:
200 mM TrisHCl (pH 8.8 at 25°C), 100 mM KCl, 100mM (NH4)2 SO4, 20 mM MgSO4, 1.0% Triton X-100

Source : E coli clone

Primers : Use 0.3 µM per primer as a general starting point. For largeramounts of template (e.g., 200 ng genomic DNA), increasing the concentration up to 0.5 µM per primer may improve yield.

Annealing Temperature:
The annealing temperature is typical PCR. The optimal annealing temperature should be ~2°C lower than the Tm of the primers used.
A range of 50–68°C is recommended.

Extension Time:
As little as 1mins per kb is suitable for most targets. Use up to 2mins per kb for maximum yield.

storage conditions : -20°C

PCR Protocol

The following procedure is suggested as a starting point when using Pfu Polymerase in any PCR amplification.
1. Add the following components to an autoclaved micro centrifuge tube at room temperature. Mix of common components to enable accurate pipetting):

Component	Volume
Pfu polymerase	0.5-1ul
10X buffer	10 ul
10mM dNTP	2 ul
Primer1 (20 pmol)	2-4 ul
Primer2 (20 pmol)	2-4 ul
template	1-10 ul
ddH2O	Up to 100 ul
Total	100 ul

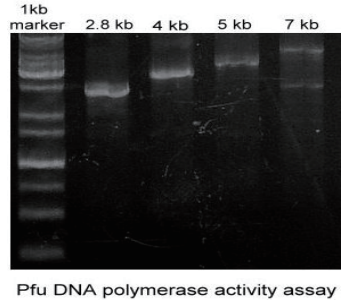
Program the thermal cycler as follows

Step	Temperature	Time	Cycle
Initial denaturation	94-95°C	1-3mins	1
Denaturation	94-95°C	0.2-1mins	25-35
Annealing	50-68°C	0.2-1mins	
Extension	68-75°C	2min/1kb	
Final extension	68-75°C	5mins	1

Step : After cycling, maintain the reaction at 4°C. Samples can be stored at –20°C until use.

Analyze products using standard agarose gel electrophoresis.

PCR result



Pfu DNA polymerase activity assay

Quality control: The enzyme is free of nicking and priming activities,exonucleases and non-specific endonucleases. SDS/PAGE - 95 kD band. Activity and s tability tested via thermo-cycling.

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