Pfu DNA polymerase

Cat No	Pack size	conc
PF0500	500 U	5U/ul
PF2500	2500 U	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 misinsertions that may occur during polymerization are rapidly excised by the proofreading activity of the polymerase. ***Pfu*** DNA polymerase is recommended for use in PCR and primer extension reactions that require high-fidelity synthesis. ***Pfu*** DNA polymerase-generated PCR fragments are blunt-ended.

Error rate: 2 x10^{-6}

Reaction Buffer (10x) with MgSO4:
200 mM TrisHCl (pH 8.8 at 25˚C), 100 mM KCl, 100 mM $(NH4)_2SO4$, 20 mM MgSO4, 1.0% Triton X-100

storage conditions: -20˚C

Unit Definition
One unit of Pfu DNA Polymerase incorporates 10 nmol of dNTP into acid-insoluble material in 30 min at 74˚C.

Primers
Use 0.3 μM per primer as a general starting point. For larger amounts 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.

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
ddH_2O	Up to 100 ul
Total	100 ul

Program the thermal cycler as follows:

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

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