

Pfu DNA Polymerase (with dNTPs), Economy

02-021 200 U 2.5U/ul, 02-021-5 200 U 2.5U/ul

Pyrococcus furiosus DNA polymerase (**Pfu** DNA polymerase) gene was expressed in E.Coli in large quantities and highly purified. The enzyme has thermostable DNA polymerase activity and $3' \rightarrow 5'$ exonuclease (proofreading) activity. The MW is 90 kDa, same as that of the natural **Pfu** DNA polymerase.

- *Pfu* DNA polymerase is thermostabe and has low error rates.
- It is suitable for PCR and primer extension reactions that require high fidelity synthesis.
- *Pfu* DNA polymerase-generated PCR fragments are blunt-ended.

Applications:

- 1) cloning
- 2) DNA expression
- 3) site-directed mutagenesis

General composition of PCR reaction mixture (total 50ul)	
Pfu DNA polymerase (2.5 units	/ul) 0.5 ul
10 x Standard Buffer (<i>Pfu</i>)	5 ul
2.5mM (each) dNTPs	4 ul
Template	<500ng
Primer 1	$0.2{\sim}1.0\mathrm{uM}$ (final conc.)
Primer 2	$0.2{\sim}1.0\mathrm{uM}$ (final conc.)
Sterile distilled water	up to $50\mathrm{ul}$

Storage Buffer:

 $50\mathrm{mM}$ Tris-HCl (pH 8.2), $0.1\mathrm{mM}$ EDTA, $1\mathrm{mM}$ DTT, 50% glycerol, 0.1% Tween20, 0.1% Igepal CA-630.

Store at:

-20°C

Concentration: 2.5 units/ul, where one unit is defined as the amount of enzyme that can incorporate 10 nmols of dNTPs into an acid-insoluble material in 30 minutes at 72°C when activated salmon sperm DNA was used as template/primer.

Quality Assurance: Greater than 95% of protein determined by SDS-PAGE (CBB staining)(Fig.1) The absence of endonucleases and exonucleases was confirmed.

PCR Test: Good amplification result was obtained in PCR reaction using \(\text{DNA} \) as a template (Fig.2).

Reagents Supplied with Enzyme:

- (1) 10 x Standard Buffer (Pfu): 200mM Tris-HCl (pH 8.8), 100mM KCl, 100mM (NH₄)₂SO₄, 20mM MgSO₄, 1% TritonX-100, 1 mg/ml BSA
- (2) 2.5mM (each) dNTPs

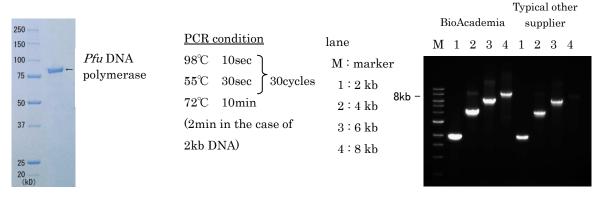


Fig.1 SDS-PAGE of Pfu DNA polymerase

Fig.2 Amplification of λ DNA