Bio Academia
Manufacturer

AS ONE

# *Pfu* DNA Polymerase (with dNTPs), Economy

# 02-021 200 U 2.5 U/µl

*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' →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 50 ul) | |
|---|---|
| *Pfu* DNA polymerase (2.5 units/ul) | 0.5 ul |
| 10 x Reaction Buffer (*Pfu*) | 5 ul |
| 2.5mM (each) dNTPs | 4 ul |
| Template | <500ng |
| Primer 1 | 0.2～1.0uM (final conc.) |
| Primer 2 | 0.2～1.0uM (final conc.) |
| Sterile distilled water | up to 50ul |

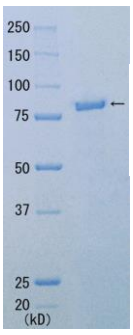
**Specification**

Storage Conditions: 50mM Tris-HCl (pH 8.2), 0.1mM EDTA, 1mM 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 λDNA as a template (Fig. 2).

**Reagents Supplied with Enzyme:**

10 x Reaction Buffer (*Pfu*): 200mM Tris-HCl (pH 8.8), 100mM KCl, 100mM $(NH_4)_2SO_4$, 20mM $MgSO_4$, 1% TritonX-100, 1 mg/ml BSA
2.5mM (each) dNTPs




*Pfu* DNA polymerase

PCR condition
98°C 10sec
55°C 30sec
72°C 10min
} 30cycles
(2min in the case of 2kb DNA)

lane
M : marker
1 : 2 kb
2 : 4 kb
3 : 6 kb
4 : 8 kb

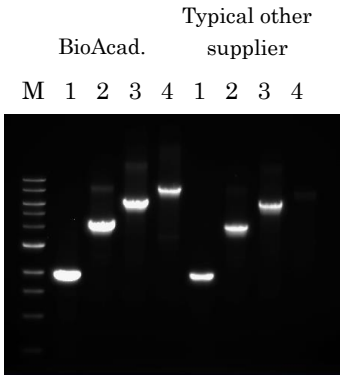



Fig. 1 SDS-PAGE of *Pfu* DNA polymerase

Fig. 2 Amplification of λ DNA