

Pfu DNA Polymerase

P0502

500 U Concentration: 2.5 U/ μ L

Store at -20°C

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' \rightarrow 3' direction. Pfu DNA Polymerase also exhibits 3' \rightarrow 5' exonuclease (proofreading) activity, that enables the polymerase to correct nucleotide incorporation errors. It has no 5' \rightarrow 3' exonuclease activity and no detectable reverse transcriptase activity. The error rate of Pfu DNA Polymerase in PCR is 2.6×10^{-6} errors per nt per cycle, as determined by a modified method described in (2).

Note.

dUTP, dITP and primers containing these nucleotides should not be used in PCR with Pfu DNA Polymerase because the binding of this enzyme to DNA templates with uracil and hypoxanthine stalls DNA synthesis (3, 4).

Applications

- High fidelity PCR
- Generation of PCR products for cloning and expression.
- RT-PCR for cDNA cloning and expression.
- Generation of PCR product for blunt-end cloning (1).
- Site-directed mutagenesis.

Source

E.coli with a cloned pol gene from *Pyrococcus furiosus*.

Definition

of Activity Unit One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30 min at 72°C. Enzyme activity is assayed in the following mixture: 20 mM Tris-HCl (pH 8.8 at 25°C), 2 mM MgSO₄, 10 mM (NH₄)₂SO₄, 10 mM KCl, 0.1 mg/mL BSA, 0.1% (v/v) Triton X-100, 0.75 mM activated calf thymus DNA, 0.2 mM of each dNTP, 0.4 MBq/mL [3H]-dTTP.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 8.2), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.1% (v/v) Nonidet P40, 0.1% (v/v) Tween 20 and 50% (v/v) glycerol. 10X Pfu Buffer with 20 mM MgSO₄ 200 mM Tris-HCl (pH 8.8 at 25°C), 100 mM (NH₄)₂SO₄, 100 mM KCl, 1 mg/mL BSA, 1% (v/v) Triton X-100, 20 mM MgSO₄.

10X Pfu Buffer:

200 mM Tris-HCl (pH 8.8 at 25°C), 100 mM (NH₄)₂SO₄, 100 mM KCl, 1% (v/v) Triton X-100, 1 mg/mL BSA.