

CERTIFICATE OF ANALYSIS

PCR buffer names have been changed.
No changes in buffer composition

Pfu DNA Polymerase

(native)

#EP0571 100u

Lot: Quality guaranteed:

Concentration: 2.5u/μl

Supplied with: 0.6ml of 10X *Pfu* Buffer with MgSO₄
0.6ml of 10X *Pfu* Buffer
0.6ml of 25mM MgSO₄

Store at -20°C

Available in certain countries only.

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BSA included:
Lot# BSA62-313P

In total 4 vials.

Description

Pfu DNA Polymerase is a DNA polymerase of *Pyrococcus furiosus*, a hyperthermophilic archaeobacterium. The enzyme catalyzes the incorporation of nucleotides into duplex DNA in the 5'→3' direction in the presence of Mg²⁺ at 70-80°C. Unlike *Taq* DNA Polymerase, *Pfu* DNA Polymerase exhibits 3'→5' exonuclease (proofreading) activity, that enables the polymerase to correct nucleotide incorporation errors.

Source

Pyrococcus furiosus cells.

Unit Definition

One unit of enzyme catalyzes the incorporation of 10 nano-moles of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30min at 72°C.

Activity Assay

20mM Tris-HCl (pH 8.8 at 25°C), 2.0mM MgSO₄, 10mM (NH₄)₂SO₄, 10mM KCl, 0.1% Triton X-100, 0.1mg/ml BSA, 0.75mM activated calf thymus DNA, 0.2mM of each dNTP, 0.4MBq/ml [³H]-dTTP.

Storage Buffer

20mM Tris-HCl (pH 8.2), 1mM DTT, 0.1mM EDTA, 100mM KCl, 0.1% Nonidet P40, 0.1% Tween 20 and 50% glycerol.

10X *Pfu* Buffer with MgSO₄

200mM Tris-HCl (pH 8.8 at 25°C), 100mM (NH₄)₂SO₄, 100mM KCl, 1% Triton X-100, 1mg/ml BSA, 20mM MgSO₄.

10X *Pfu* Buffer

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

Applications

- All PCR applications which demand high fidelity.
- High fidelity PCR for cloning into blunt-ended vectors (1), see enclosed protocol.
- Site-directed mutagenesis.

Note

- The optimal reaction conditions (incubation time and temperature, concentration of *Pfu* DNA Polymerase, template DNA, MgSO₄) depend on the template-primer pair and must be determined individually. It is especially important to titrate the MgSO₄ concentration and the amount of enzyme required per assay. The standard concentration of MgSO₄ is 2mM and amount of *Pfu* DNA Polymerase is 1.25u per 50µl of reaction mixture.
- *Pfu* DNA Polymerase remains 95% active after 2 hours incubation at 95°C.
- The error rate of *Pfu* DNA Polymerase in PCR is 2.6×10^{-6} errors per nt per cycle; the accuracy (an inverse of error rate) an average number of correct nucleotides incorporated before making an error – is 3.8×10^5 (determined according to the modified method described in (2)).
- *Pfu* DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The enzyme has no detectable reverse transcriptase activity.
- Do not use dUTP in PCR.

QUALITY CONTROL

Endodeoxyribonuclease Assay

No detectable degradation of lambda DNA was observed after incubation of 10 units of *Pfu* DNA Polymerase with 1µg DNA in 50µl of *Pfu* buffer with MgSO₄ containing 0.2mM of each dNTP for 4 hours at 72°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA/HindIII fragments was observed after incubation of 10 units of *Pfu* DNA Polymerase with 1µg digested DNA in 50µl of *Pfu* buffer with MgSO₄ containing 0.2mM of each dNTP for 4 hours at 72°C.

Functional Assay

Pfu DNA Polymerase was tested for amplification of a 950 bp single copy gene from human genomic DNA.

Quality authorized by:



Jurgita Zilinskiene

(continued on back page)

References

1. Sambrook, J., Russel, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
2. Lundberg, K.S., et al., High-fidelity amplification using a thermostable DNA polymerase isolated from *Pyrococcus furiosus*, Gene, 108, 1-6, 1991.

Related Products

- 2mM dNTP Mix #R0241, #R0242
- 10mM dNTP Mix #R0191, #R0192
- dNTP Set #R0181, #R0182, #R0186
- Modified Nucleotides #R0081, #R0091, #R0101, #R0111, #R0121
- FastRuler™ DNA Ladders #SM1103, #SM1113, #SM1123
- O'RangeRuler™ DNA Ladders #SM0613, #SM0623, #SM0633, #SM643, #SM653
- GeneRuler™ DNA Ladders #SM0241, #SM0242, #SM0243 #SM0321, #SM0322, #SM0323
- Φ X174 DNA/BsuRI Marker, 9 #SM0251, #SM0252, #SM0253

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Tween is a registered trademark of ICI America, Inc.

Triton X-100 is a registered trademark of Rohm&Haas, Inc.

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