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LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Pfu DNA Polymerase
(recombinant)

#EP0501 100u

Lot: **Expiry Date:**

Concentration: 2.5u/µl

Supplied with: 0.6ml of 10X *Pfu* Buffer with $MgSO_4$
0.6ml of 10X *Pfu* Buffer
0.6ml of 25mM $MgSO_4$

Store at -20°C

Available in certain countries only.

In total 4 vials. BSA included: Lot# BSA62-313P

ISO 9001 ISO 14001
www.fermentas.com

Description

Pfu DNA Polymerase is a DNA polymerase of *Pyrococcus furiosus,* a hyperthermophillic archaebacterium. The enzyme catalyzes the incorporation of nucleotides into duplex DNA in the 5'→3' direction in the presence of Mg^{2+} 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

E.coli cells carrying a cloned *pol* gene from *Pyrococcus furiosus*.

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 $MgSO_4$, 10mM $(NH_4)_2SO_4$, 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 [^{3}H]-dTTP.

Storage Buffer

Enzyme is supplied in: 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 $MgSO_4$

200mM Tris-HCl (pH 8.8 at 25°C), 100mM $(NH_4)_2SO_4$, 100mM KCl, 1% Triton X-100, 1mg/ml BSA, 20mM $MgSO_4$.

10X Pfu Buffer

200mM Tris-HCl (pH 8.8 at 25°C), 100mM $(NH_4)_2SO_4$, 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, $MgSO_4$) depend on the template-primer pair and must be determined individually. It is especially important to titrate the $MgSO_4$ concentration and the amount of enzyme required per assay. The standard concentration of $MgSO_4$ 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 $MgSO_4$ 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 $MgSO_4$ 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.

License

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
- ΦX174 DNA/BsuRI Marker, 9 #SM0251, #SM0252, #SM0253

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

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