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CERTIFICATE OF ANALYSIS

Taq DNA Polymerase (recombinant)

#EP0402 500 u

Lot: **Expiry Date:**

Concentration: 5 u/µl
Supplied with: 2x1.25 ml of 10X *Taq* Buffer with KCl
2x1.25 ml of 10X *Taq* Buffer with $(NH_4)_2SO_4$
2x1.25 ml of 25mM $MgCl_2$

Store at –20°C

In total 7 vials.

ISO 9001 ISO 14001
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Description

Taq DNA Polymerase is a highly thermostable DNA polymerase of a thermophilic bacterium *Thermus aquaticus.* *Taq* DNA Polymerase catalyzes 5'→3' synthesis of DNA. The enzyme has no detectable 3'→5' proofreading exonuclease activity and possesses low 5'→3' exonuclease activity.

Source

E.coli cells carrying a cloned *pol* gene from *Thermus aquaticus.*

Unit Definition

One unit of enzyme catalyzes the incorporation of 10 nanomoles of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30min at 70°C.

Activity Assay

67mM Tris-HCl (pH 8.8 at 25°C), 6.7mM $MgCl_2$, 1mM 2-mercaptoethanol, 50mM NaCl, 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 8.0), 1mM DTT, 0.1mM EDTA, 100mM KCl, 0.5% Nonidet P40, 0.5% Tween 20 and 50% glycerol.

10X Taq Buffer with KCl

100mM Tris-HCl (pH 8.8 at 25°C), 500mM KCl, 0.8% Nonidet P40.

10X Taq Buffer with $(NH_4)_2SO_4$

750mM Tris-HCl (pH 8.8 at 25°C), 200mM $(NH_4)_2SO_4$, 0.1% Tween 20.

Applications

- PCR amplification of DNA fragments as long as 5 kb (1), *see* the enclosed Protocol.
- DNA labeling (2-4).
- DNA sequencing (5).
- PCR for cloning.

Note

- Recombinant *Taq* DNA Polymerase is the enzyme of choice for most PCR applications.
- The half-life of enzyme is >40 minutes at 95°C.
- Both *Taq* buffers can be used for the same applications. However, the higher and more consistent yield of the specific PCR product over a wide range of $MgCl_2$ concentration can be achieved in the buffer with $(NH_4)_2SO_4$ than in the traditional buffer.
- The error rate of *Taq* DNA Polymerase in PCR is $2.2x10^{-5}$ errors per nt per cycle; the accuracy (an inverse of the error rate) an average number of correct nucleotides incorporated before making an error, is $4.5x10^4$ (determined according to the modified method described in (6)).
- *Taq* DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 10 units of *Taq* DNA Polymerase with 1µg of pBR322 DNA in 50µl of *Taq* Buffer with KCl containing 1.5mM $MgCl_2$ for 4 hours at 37°C.

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 10 units of *Taq* DNA Polymerase with 1µg of pBR322 DNA in 50µl of *Taq* Buffer with KCl containing 1.5mM $MgCl_2$ for 4 hours at 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA/HindIII fragments was observed after incubation of 10 units of *Taq* DNA Polymerase with 1µg of digested DNA in 50µl of *Taq* Buffer with KCl containing 1.5mM $MgCl_2$ for 4 hours at 37°C.

No detectable degradation of lambda DNA/HindIII fragments was observed after incubation of 10 units of *Taq* DNA Polymerase with 1µg of digested DNA in 50µl of *Taq* Buffer with KCl containing 1.5mM $MgCl_2$ for 4 hours at 70°C.

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Ribonuclease Assay

0.2% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10 units of *Taq* DNA Polymerase with 1µg of [^{3}H]-RNA in 50µl of *Taq* Buffer with KCl containing 1.5mM $MgCl_2$ for 4 hours at 37°C.

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10 units of *Taq* DNA Polymerase with 1µg of [^{3}H]-RNA in 50µl of *Taq* Buffer with KCl containing 1.5mM $MgCl_2$ for 4 hours at 70°C.

Functional Assay

Taq DNA Polymerase was tested for amplification of 950 bp single copy gene from human genomic DNA and for amplification of cDNA.

Quality authorized by: Jurgita Zilinskiene

DE-81 – Whatman anion exchange chromatography paper having diethylaminoethyl functional groups.
Whatman is a registered trademark of Whatman Ltd.
Nonidet is a registered trademark of Shell.
Tween is a registered trademark of ICI America, Inc.

References

1. Innis, M.A., et al., PCR Protocols and Applications: A Laboratory Manual, Academic, New York, 1989.
2. Celeda, D., et al., PCR amplification and simultaneous digoxigenin incorporation of long DNA probes for fluorescence *in situ* hybridization, BioTechniques, 12, 89-102, 1992.
3. Finckh, U., et al., Producing single-stranded DNA probes with the *Taq* DNA polymerase: a high yield protocol, BioTechniques, 10, 35-39, 1991.
4. Yu, H., et al., Cyanine dye dUTP analogs for enzymatic labeling of DNA probes, Nucleic Acids Res., 22, 3226-3232, 1994.
5. Innis, M.A., et al., DNA sequencing with *Thermus aquaticus* DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA, Proc. Natl. Acad. Sci. USA, 85, 9436-9440, 1988.
6. Lundberg, K.S., et al., High-fidelity amplification using a thermostable DNA polymerase isolated from *Pyrococcus furiosus*, Gene, 108, 1-6, 1991.

Related Products

- 2X PCR Master Mix #K0171
- 2mM dNTP Mix #R0241, #R0242
- dNTP Set #R0181, #R0182, #R0186
- Modified Nucleotides #R0081, #R0091, #R0101, #R0111, #R0121
- InsT/Aclone™ PCR Cloning Kit #K1213, #K1214
- 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
- 10X *Taq* Buffers with KCl Set #B15

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