

CERTIFICATE OF ANALYSIS

***Taq* DNA Polymerase**
(native, without BSA)

#EP0282 500u

Lot: **Expiry Date:**

Concentration: 5u/μl
Supplied with: 2x1.25ml of 10X *Taq* Buffer with KCl
 2x1.25ml of 10X *Taq* Buffer with (NH₄)₂SO₄
 2x1.25ml of 25mM MgCl₂

Store at -20°C

Description

Taq DNA Polymerase is a highly thermostable DNA polymerase of a thermophilic bacterium *Thermus aquaticus*. The enzyme catalyzes 5'→3' synthesis of DNA. It has no detectable 3'→5' proofreading exonuclease activity, and possesses low 5'→3' exonuclease activity. Native *Taq* DNA Polymerase is preferred for amplifications of bacterial DNA sequences homologous to those found in *E.coli*.

Source

Thermus aquaticus YT1 cells.

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 MgCl₂, 1mM 2-mercaptoethanol, 50mM NaCl, 0.1mg/ml BSA, 0.75mM activated calf thymus DNA, 0.2mM of each dNTP, 0.4MBq/ml [³H]-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 (NH₄)₂SO₄

750mM Tris-HCl (pH 8.8 at 25°C), 200mM (NH₄)₂SO₄, 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

- 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 MgCl₂ concentration can be achieved in the buffer with (NH₄)₂SO₄ 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 4.5x10⁴ (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 MgCl₂ 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 MgCl₂ for 4 hours at 70°C.

Ribonuclease Assay

0.8% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10 units of *Taq* DNA Polymerase with 1µg of [³H]-RNA in 50µl of *Taq* buffer with KCl containing 1.5mM MgCl₂ 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

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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.

DE-81 – Whatman anion exchange chromatography paper having diethylaminoethyl functional groups.

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Related Products

- 2X PCR Master Mix #K0171
- 2mM dNTP Mix #R0241, #R0242
- dNTP Set #R0181, #R0182, #R0186
- Modified Nucleotides #R0081, #R0091, #R0101, #R0111, #R0121
- PCR Optimization Kit #K0162
- InsT/Aclone PCR Product 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
- Φ X174 DNA/BsuRI Marker, 9 #SM0251, #SM0252, #SM0253
- 10X *Taq* Buffers with KCl Set #B15

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