

CERTIFICATE OF ANALYSIS

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

# Taq DNA Polymerase (recombinant)

#EP0401 100u

Lot: Quality guaranteed:

Concentration: 5u/ $\mu$ l  
Supplied with: 0.6ml of 10X Taq Buffer with KCl  
0.6ml of 10X Taq Buffer with  $(\text{NH}_4)_2\text{SO}_4$   
0.6ml of 25mM  $\text{MgCl}_2$

Store at  $-20^\circ\text{C}$

3

In total 4vials.

## 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  $70^\circ\text{C}$ .

## Activity Assay

67mM Tris-HCl (pH 8.8 at  $25^\circ\text{C}$ ), 6.7mM  $\text{MgCl}_2$ , 1mM 2-mercaptoethanol, 50mM NaCl, 0.1mg/ml BSA, 0.75mM activated calf thymus DNA, 0.2mM of each dNTP, 0.4MBq/ml [ $^3\text{H}$ ]-dTTP.

## Storage Buffer

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  $25^\circ\text{C}$ ), 500mM KCl, 0.8% Nonidet P40.

## 10X Taq Buffer with $(\text{NH}_4)_2\text{SO}_4$

750mM Tris-HCl (pH 8.8 at  $25^\circ\text{C}$ ), 200mM  $(\text{NH}_4)_2\text{SO}_4$ , 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<sub>2</sub> concentration can be achieved in the buffer with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> than in the traditional buffer.
- The error rate of *Taq* DNA Polymerase in PCR is 2.2x10<sup>-5</sup> 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<sup>4</sup> (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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> for 4 hours at 70°C.

*(continued on back page)*

## ***Ribonuclease Assay***

0.1% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10 units of *Taq* DNA Polymerase with 1 µg of [<sup>3</sup>H]-RNA in 50 µl of *Taq* Buffer with KCl containing 1.5mM MgCl<sub>2</sub> 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 1 µg of [<sup>3</sup>H]-RNA in 50 µl of *Taq* Buffer with KCl containing 1.5mM MgCl<sub>2</sub> 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
- 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
- ΦX174 DNA/BsuRI Marker, 9 #SM0251, #SM0252,  
#SM0253
- 10X *Taq* Buffers with KCl Set #B15

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