

**CERTIFICATE OF ANALYSIS**

**Taq DNA Polymerase**  
(native, with BSA)

**#EP0072**      500u

**Lot:**                      **Expiry Date:**

Concentration:      5u/μl  
Supplied with:      2x1.25ml of 10X *Taq* Buffer with KCl  
                                 2x1.25ml of 10X *Taq* Buffer with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
                                 2x1.25ml of 25mM MgCl<sub>2</sub>  
                                 0.25ml (2mg) of 20mg/ml BSA

**Store at -20°C**

In total 8 vials.

BSA included: Lot# BSA62-313P

**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. *Taq* DNA Polymerase (native, with BSA) is preferred for amplifications of bacterial DNA sequences homologous to those found in *E.coli*. It is often the best choice when amplifying DNA samples of lower purity.

**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 MgCl<sub>2</sub>, 1mM 2-mercaptoethanol, 50mM NaCl, 0.1mg/ml BSA, 0.75mM activated calf thymus DNA, 0.2mM of each dNTP, 0.4MBq/ml [<sup>3</sup>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, 0.2mg/ml BSA 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 $(\text{NH}_4)_2\text{SO}_4$**

750mM Tris-HCl (pH 8.8 at 25°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**

- The recommended concentration of BSA in the reaction mixture is 0.1mg/ml.
- 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  $\text{MgCl}_2$  concentration can be achieved in the buffer with  $(\text{NH}_4)_2\text{SO}_4$  than in the traditional buffer.
- The error rate of *Taq* DNA Polymerase in PCR is  $2.2 \times 10^{-5}$  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  $4.5 \times 10^4$  (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  $\text{MgCl}_2$  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  $\text{MgCl}_2$  for 4 hours at 70°C.

### **Ribonuclease Assay**

0.4% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10 units of *Taq* DNA Polymerase with 1µg of [<sup>3</sup>H]-RNA in 50µl of *Taq* buffer with KCl containing 1.5mM  $\text{MgCl}_2$  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

(continued on back page)

## 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.

**Whatman** is a registered trademark of Whatman Ltd.

**Nonidet** is a registered trademark of Shell.

**Tween** is a registered trademark of ICI America, Inc.

## 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
- $\Phi$ X174 DNA/BsuRI Marker, 9 #SM0251, #SM0252, #SM0253
- 10X *Taq* Buffers with KCl Set #B15

### **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](http://www.fermentas.com) for Material Safety Data Sheet of the product.

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