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## Certificate of Analysis

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## Human DNA Polymerase Beta

Catalog No: 1077

**Lot No:** See Product Label

**Package Size:** See Product Label

**Concentration:** See Product Label

**Protein (Bradford):** 1.167 mg/ml

**Specific Activity:** 4,367 units/mg

**Storage Conditions:** Store at -80°C

**Notes:** Avoid multiple freeze / thaw cycles or frequent temperature changes

### Description

- Simplest DNA polymerase known in both size and catalysis (1)

### Applications

- Repair polymerase able to synthesize DNA beyond the end of a gap or nick with simultaneous displacement of the non-replicated strand (2)
- Fills gaps or nicks (3)

### Unit Definition

One unit is the amount of enzyme required to incorporate 1 nmol of total nucleotide into acid-insoluble form in 60 minutes at 37°C

### Assay Conditions

50 mM Tris-HCl (pH 8.7)

10 mM KCl

10 mM MgCl<sub>2</sub>

0.4 mg/ml bovine serum albumin

1 mM dithiothreitol

0.05 mM of each dCTP, dGTP, dTTP, and [ $\alpha$ -<sup>32</sup>P]dATP

15% (v/v) glycerol

10  $\mu$ g activated DNA

Reaction volume of 50  $\mu$ l

### Storage Buffer

20 mM Tris-HCl (pH 8.0)

0.1 mM EDTA

200 mM NaCl

1 mM dithiothreitol

50% (v/v) glycerol

### Quality Control

**SDS-PAGE Gel:** Approximately 99% pure, as judged by silver stain SDS-polyacrylamide gel electrophoresis.

**Nicking:** Incubation of 2.5, 5.0, and 10.0 units of enzyme with 1.0  $\mu$ g of pBR322 DNA at 37°C for 1 hour resulted in  $\leq$ 10% conversion of RFI to RFII. Reaction volume of 50  $\mu$ l.

### 5'-Exonuclease/5'-Phosphatase:

Incubation of 2.5, 5.0, and 10.0 units of enzyme with 0.25  $\mu$ g of 5'-ends of [5'-<sup>32</sup>P]lambda/Hae III DNA fragments for 1 hour at 37°C resulted in a  $\leq$ 0.4 slope of %-end label released per unit of enzyme. Reaction volume of 50  $\mu$ l.

**3'-Exonuclease:** Incubation of 2.5, 5.0, and 10.0 units units of enzyme with 5 pmoles of 3'-ends of lambda/Taq I fragments (3'-labeled with Klenow DNA Polymerase and [<sup>3</sup>H]dCTP, incubated for 1 hour at 37°C resulted in a  $\leq$ 0.4 slope of %-end label released per unit of enzyme. Reaction volume of 50  $\mu$ l.

### References

- (1) Abbotts, J., SenGupta, D.N., Zmudzka, B., Widen, S.G., Notario, V., and Wilson, S.H. (1988) *Biochemistry* 27, No. 3, 901-909
- (2) Nowak, R., Kulik, J., and Siedlecki, J.A. (1987) *Acta Biochim Pol.* 34, 205-215
- (3) Wang, T.S.F., and Korn, D. (1980) *Biochemistry* 19, 1782-1790

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