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Certificate of Analysis

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DNA Polymerase I

Catalog No: 1080

Lot No: See Product Label

Package Size: See Product Label

Concentration: See Product Label

Protein (Bradford): 0.964 mg/ml

Specific Activity: 5,185 units/mg

Storage Conditions: Store at -20°C

Storage Buffer

20 mM Tris-HCl (pH 8.0)

0.1 mM EDTA

200 mM NaCl

1 mM dithiothreitol

50% (v/v) glycerol

Description

- Exhibits the 5'→3' polymerase activity
- Exhibits the 5'→3' exonuclease activity, active only on duplex DNA
- Contains the 3'→5' exonuclease, primarily active on single stranded DNA (1)
- Ultrapure recombinant enzyme

Applications

- Used to prepare radioactive probes by nick translation (2) and random priming (3)
- Useful for end-labeling of DNA molecules with 3' and 5' protruding tails or blunt-ended

Unit Definition

One unit is the amount of enzyme required to incorporate 10 nmoles of total deoxyribonucleotide into acid-insoluble material in 30 minutes at 37°C with DNase I-activated DNA as the template primer.

Assay Conditions

67 mM potassium phosphate (pH 7.4)

6.7 mM MgCl₂

1 mM dithiothreitol

0.033 mM of each dCTP, dGTP, dTTP and [α -³²P] dATP

4.5 μ g activated DNA

Incubation is for 30 minutes at 37°C in a total reaction volume of 100 μ l

Quality Control

Nicking: Incubation of 5, 10 and 20 units of DNA Polymerase with 0.5 μ g of pBR322 DNA at 37°C for 1 hour resulted in \leq 10% conversion of RFI to RFII DNA and \leq 1% RF III. Reaction volume of 50 μ l.

Purity: Approximately \geq 95% pure, as judged by SDS-polyacrylamide gel electrophoresis

References

(1) Lehman, I.R., *Enzymes* 14, 15-37. 1981 (2) Rigby, P.W.J., Diekmann, M., Rhodes, C. and Berg, P., *J. Mol. Biol.* 113, 237-251. 1977 (3) Hartman, C.P. and Robussay, D., *Gene Amplification and Analysis* (Chirikjian, J.G. and Pappas, T.S., eds.) 2, 17-39, Elsevier/North Holland, New York. 1981