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Certificate of Analysis

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Klenow Fragment

Catalog No: 1091

Lot No: See Product Label

Package Size: See Product Label

Concentration: See Product Label

Protein (Bradford): 1.533 mg/ml

Specific Activity: 6,519 units/mg

Storage Conditions: Store at -20°C

Description

- Lacks 5' exonuclease activity (1)
- Ultrapure recombinant enzyme

Applications

- Used for Sanger dideoxy sequencing (2)
- Suitable for second strand cDNA synthesis (3)
- Used for 3'-end labeling and filling in 5'-protruding sticky ends (4)

Unit Definition

One unit is the amount of enzyme required to incorporate 10 nmoles of total nucleotide into acid insoluble form in 30 minutes at 37°C

Assay Conditions

67 mM potassium phosphate (pH 7.4)

6.7 mM MgCl₂

1.0 mM dithiothreitol

0.033 mM [α -³²P] dATP

0.033 mM dCTP

0.033 mM dGTP

0.033 mM dTTP

4.5 μ g activated DNA

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

Storage Buffer

50 mM potassium phosphate (pH 7.0)

0.25 mM dithiothreitol

50% glycerol.

Quality Control

Endonuclease: Incubation of 5, 10, and 20 units of Klenow fragment with 1.0 μ g of pBR322 DNA at 37°C for 1 hour resulted in \leq 10% conversion of RFI to RFII DNA and \leq 5% RF III. Reaction volume of 50 μ l

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

References

(1) Joyce, C.M. and Grindley, N.D., *Proc. Natl. Acad. Sci. USA* 80, 1830-1834. 1983 (2) Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463-5467 (3) Houdebine, L.M. (1976) *Nucleic Acids Res.* 3, 615-630 (4) Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Mol. Cloning: A Laboratory Manual, Second Ed.* pp. 5.34, 5.4-5.43 (5) Richardson, C.C., Schildkraut, C.L., Aposhian, H.V. and Kornberg, A., *J. Biol. Chem.* 239, 222-232. 1964