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Certificate of Analysis

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Klenow Fragment, Exonuclease Free

Catalog No: 1092

Lot No: See Product Label
Package Size: See Product Label
Concentration: See Product Label
Protein (Bradford): 1.44 mg/ml
Specific Activity: 46,943 units/mg
Storage Conditions: Store at -20°C

Description

- Lacks both 3'→5' exonuclease and 5'→3' exonuclease activities (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 nmol 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 $MgCl_2$
1.0 mM dithiothreitol
0.033 mM [α-^{32}P]dATP
0.033 mM dCTP
0.033 mM dGTP
0.033 mM dTTP
4.5 µg activated DNA
Incubation is at 37°C for 30 minutes in a reaction volume of 100 µl.

Storage Buffer

50 mM potassium phosphate (pH 7.0) 0.25 mM dithiothreitol

50% glycerol

Quality Control

3'-Exonuclease: Incubation of 10, 20, and 40 units of Klenow Fragment (Exonuclease Free) and 5 pmoles of 3'-ends of lambda/Taq I fragments (3'-labeled with Klenow exo- and [^{3}H]dCTP), incubated for 1 hour at 37°C resulted in ≤1.0 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

5'-Exonuclease/5'-Phosphatase: Incubation of 10, 20, and 40 units of Klenow Fragment (Exonuclease Free) with 0.25 µg of 5'-ends of [5'-^{32}P]lambda/Hae III fragments for 1 hour at 37°C resulted in ≤1.0 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

Nicking: Incubation of 10, 20, and 40 units of Klenow Fragment (Exonuclease Free) with 1µgof pBR322 DNA at 37°C for 1 hour resulted in ≤5% of RFI to RFII DNA. Reaction volume of 50 µl.

Purity: ≥ 90% pure, as judged by SDS-polyacrylamide gel electrophoresis

References

(1) Derbyshire, V., Freemont, P.S., Sanderson, M.R., Beese, L., Friedman, J.M., Joyce, C.M. and Steitz, T.A. (1988) Science 240, 199-201 (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