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Certificate of Analysis

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T4 Polynucleotide Kinase

Catalog No: 1261

Lot No: See Product Label
Package Size: See Product Label
Concentration: See Product Label
Storage Conditions: Store at -20°C

Applications

- Catalyzes the transfer of the gamma-phosphate of ATP to the 5'-OH terminus in DNA or RNA
- Contains 3'-phosphatase activity (1)
- Used for 5'-end labeling of nucleic acids prior to DNA or RNA sequencing (2, 3)
- Phosphorylates synthetic linkers and fragments of DNA or RNA prior to ligation
- Labels 5'-termini prior to partial restriction enzyme digestion

Unit Definition

One unit is the amount of enzyme required to transfer 1 nmol of γ-phosphate from ATP to the 5'-OH terminus of salmon sperm DNA in 30 minutes at 37°C.

Assay Conditions

70 mM Tris-HCl (pH 7.6)
10 mM $MgCl_2$
5 mM dithiothreitol
27 nmol DNA-phosphorus (5'-OH terminated salmon sperm DNA)
70 mM[γ-^{32}P]ATP
Reaction volume of 100 µl

Storage Buffer

50 mM Tris-HCl (pH 7.6)
0.1 mM EDTA
25 mM KCl
1 mM dithiothreitol
0.1 µM ATP
50% glycerol

Quality Control

Nicking: Incubation of 10, 20, and 40 units of enzyme with 1.0 µg of pBR322 DNA at 37°C for 1 hour resulted in ≤10% conversion of RFI to RFII. Reaction volume of 50 µl.

3'-Exonuclease: Incubation of 5, 10, and 20 units of enzyme with 5 pmoles of 3'-ends of lambda/Taq I fragments (3'-labeled with Klenow exo- and [^{3}H]dCTP), for 1 hour at 37°C resulted in a ≤0.4 slope of %- end label released per unit of enzyme. Reaction volume of 50 µl.

RNase: Incubation of 10, 20, and 40 units of enzyme with 0.015 µg of [^{32}P] RNA transcript for 1.0 hour at 37°C resulted in a ≤0.4 slope of %-end label released per unit of the labeled substrate. Reaction volume of 50 µl.

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

References

(1) Richardson, C.C. (1971) Progress in Nucleic Acids Res. and Mol. Biol. 2, 815-828 (2) Maxam, A. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560-56 (3) Donis-Keller, H. (1980) Nucleic Acids Res. 8, 3133-3142