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Certificate of Analysis

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T4 DNA Polymerase

Catalog No: 1100

Lot No: See Product Label
Package Size: See Product Label
Concentration: See Product Label
Protein: 0.131 mg/ml
Specific Activity: 76,336 units/mg
Storage Conditions: Store at -20°C

Description

- Exhibits 5'→3' polymerase and 3'→5' exonuclease activities (1, 2)
- Requires the presence of a single-stranded DNA template and a primer
- Exonuclease, stronger than that found in DNA Polymerase I, is more active on single-stranded DNA than on double-stranded DNA
- Ultrapure recombinant enzyme

Applications

- Adds labeled nucleotides to the recessed 3'-ends of DNA fragments Exonuclease activity can be used to remove one or a few nucleotides from 3'-end of double–stranded DNA
- Enzyme suitable for:
  -3' overhang removal to form blunt ends (3,4)
  -5' fill-in to form blunt ends (3,4)
  -Probe labeling using replacement synthesis (3,4)
  -Single strand deletion subcloning (5)
  -Second strand synthesis in site-directed mutagenesis (6)

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 8.8)
6.7 mM $MgCl_2$
1.0 mM dithiothreitol
16.6 mM ammonium sulfate
6.7 µM EDTA
167 µg/ml bovine serum albumin
0.033 mM [α-^{32}P] dATP
0.033 mM dCTP
0.033 mM dGTP
0.033 mM dTTP
45 µg activated DNA. Incubation is at 37°C for 30 minutes in a reaction volume of 100 µl

Storage Buffer

20 mM potassium phosphate (pH 7.5)
5 mM dithiothreitol
50% (v/v) glycerol

Quality Control

Endonuclease: Incubation of 5, 10, and 20 units of T4 DNA Polymerase with 1.0 µg of pBR322 DNA at 37°C for 1 hour resulted in ≤10% conversion of RFI to RFII DNA. Reaction volume of 50 µl.

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

References

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