DNA Polymerase I (E.coli)

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DNA Polymerase I (Escherichia coli)

Source: Escherichia coli

DNA Polymerase I is a mezophilic, DNA-dependent DNA polymerase with inherent 3'-> 5' and 5'-> 3' exonuclease activity.

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

Storage Conditions: Store at -20°C.

Storage Buffer: 50 mM potassium phosphate (pH 7.0), 0.25 mM dithiothreitol and 50% (v/v) glycerol.

Assay Conditions: 67 mM potassium phosphate (pH 7.4), 6.7 mM $MgCl_2$, 1 mM dithiothreitol, 0.033 mM each dCTP, dGTP, dTTP and [α-^{32}P]dATP, 4.5 µg activated DNA. Incubation is at 37°C for 30 min in a reaction volume of 100 µl.

Quality Control: All preparations are assayed for contaminating endonuclease activity. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis.

References:

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