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ProductInformation

DNA Polymerase I
From *E. coli* lysogen carrying bacteriophage
Product Number **D9380**
Storage Temperature –20 °C

CAS# 9012-90-2
EC 2.7.7.7
Synonym: Kornberg polymerase

Product Description

Molecular weight: 109 kDa

DNA polymerase I (holoenzyme) has 5'→3' and 3'→5' exonuclease activities in addition to its synthetic activity. This bifunctional activity enables the enzyme to use nicks or gaps in double stranded DNA as starting points for DNA synthesis. The 5'→3' exonuclease activity degrades the DNA strand complementary to the template strand beginning at the nick. DNA synthesis begins at the 3'-end of the nick and produces a new strand of DNA complementary to the template. The net result is the movement of the polymerase along the template strand (nick translation) until the DNA complementary to the template (from the site of the original nick to the 5'-end of the template strand) is replaced.

The enzyme may be used with radioactive or biotinylated nucleotides to prepare labeled DNA of high specific activity by nick translation,[1] for *in vitro* synthesis of complementary cDNA strand,[2] for *in vitro* synthesis of DNA, and to produce blunt ends from 5' and 3' overhangs.[3]

Concentration: 5,000-15,000 units/ml

Unit definition: One unit converts 10 nanomoles of deoxyribonucleoside triphosphates into acid insoluble material in 30 min at 37 °C.

Endonuclease: none detected.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To ensure recovery of product from its storage container, centrifuge prior to use.

Storage/Stability

Store at –20 °C. DNA polymerase I is provided as a solution in 50% glycerol containing 100 mM potassium phosphate buffer, pH 6.5, and 1 mM dithiothreitol. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended.

Procedure

Unit Assay Conditions:
40 mM potassium phosphate, pH 7.5, 6.6 mM $MgCl_2$, 1mM 2-mercaptoethanol, 20 µM poly[dA] • poly[dT], (Product No. P9764), 33 µM dATP (Product No. D4758), 33 µM ^{3}H-TTP, and 0.05-0.1 units DNA Polymerase.

References

1. Meinkoth, J., and Wahl, G.M., Nick translation. *Meth. Enzymol.* **152**, 91-94 (1987).
2. Gubler, U., *et al.*, A simple and very efficient method for generating cDNA libraries. *Gene,* **25**, 263-269 (1983).
3. Okayama, H., and Berg, P., High-efficiency cloning of full-length cDNA. *Mol. Cell. Biol.,* **2**, 161-170 (1982).
4. D'Alessio, J.M., and Gerard, G.F., Second-strand cDNA synthesis with *E. coli* DNA polymerase I and RNase H: the fate of information at the mRNA 5' terminus and the effect of *E. coli* DNA ligase. *Nucl. Acids Res.,* **16**, 1999-2014 (1988).

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