

Product Information

DNA Polymerase I, Klenow Fragment from *Escherichia coli*

Catalog Number **D8276**Storage Temperature -20°C

CAS RN 9012-90-2

Product Description

DNA polymerase I from *E. coli* yields two fragments (small and large) upon protease digestion. The large fragment (Klenow fragment) loses the 5' exonuclease activity that is present in the intact holoenzyme. However, it retains both the polymerase 5'→3' activity and the 3'→5' exonuclease activity of the native enzyme.

This gives the Klenow fragment a number of applications:

1. DNA sequencing by the Sanger dideoxy method
2. Generation of the complementary strand of cDNA
3. Extensions by "fill in" of 5'-overhangs in double stranded DNA to form blunt ends
4. Mutagenesis of DNA with second strand synthesis using oligonucleotides
5. Labeling DNA by the random primer method

The product is supplied in a solution of 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 5 mM dithiothreitol, and 50% (v/v) glycerol.

Concentration: ~3,000 units/mL

Unit definition: One unit converts 10 nanomoles of deoxyribonucleoside triphosphates into acid insoluble material in 30 minutes at 37°C . The activity is assayed in a reaction mixture containing 50 mM potassium phosphate, pH 7.5, 3 mM MgCl_2 , 32.5 μM ^{32}P -dATP, 1 mM 2-mercaptoethanol, 32.5 μM dTTP, 62.5 $\mu\text{g/ml}$ poly(dA-dT), and 0.02–1 unit of enzyme.

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

The enzyme solution may be diluted with 50 mM Tris-HCl, pH 7.5, 100 mM ammonium sulfate, 10 mM 2-mercaptoethanol, and 1 mg/ml bovine serum albumin.

Storage/Stability

The product ships on wet ice and storage at -20°C is recommended. Do not store in a frost-free freezer. The frequent freeze-thaw cycles may decrease enzyme activity.

References

1. Sanger, F. *et al.*, Proc. Natl. Acad. Sci. USA, **74**, 5463 (1977).

BR,KH,JB,MAM 05/08-1

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