

Klenow Fragment, 3'→5' exo⁻

DNA Polymerase I

recombinant, *E. coli*

Cat.-No.	Amount	Conc.
EN-151S	200 units	10 units/μl
EN-151L	1000 units	10 units/μl

For *in vitro* use only
Quality guaranteed for 12 months
Store at -20 °C

Avoid freeze / thaw cycles

Description

The Klenow Fragment is a proteolytic product of *E. coli* DNA polymerase I that catalyzes the 5'→3' synthesis of DNA but has lost the 5'→3' exonuclease activity. The exonuclease-deficient variant is genetically engineered to abolish its 3'→5' exonuclease activity. The enzyme is suited for second strand synthesis, particularly for synthesis of DNA using fluorescent nucleotide analogs.

Standard DNA Polymerase Assay Conditions

The polymerase activity is assayed in 1x Klenow Fragment Reaction Buffer including 300 μM dNTPs, M13mp18 ss-DNA and M13 universal primer. Quantification of ds-DNA is performed using PicoGreen[®].

Absence of contaminants

Tested for the absence of endo- and exodeoxy-ribonucleases.

Klenow Fragment

Klenow Fragment in 100 mM KH₂PO₄/K₂HPO₄ (pH 6.5), 1 mM DTT and 50% glycerol.

10x Klenow Reaction Buffer

100 mM Tris-HCl (pH 7.5), 50 mM MgCl₂, 75 mM DTT

Activity

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmoles of dNTPs into acid-insoluble material in 30 minutes at 37 °C.

Purity

>95% by SDS PAGE

Selected references

Brakmann S. and Löbermann S. (2001) High-Density Labeling of DNA: Preparation and Characterization of the Target Material for Single-Molecule Sequencing. *Angew. Chem. Int. Ed.* **40**:1427.

Tveit H. and Kristensen T. (2001) Fluorescence-based DNA polymerase assay. *Anal. Biochem.* **289**:96.

Sanger et al. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463.

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