



Klenow Fragment

Large Fragment of DNA Polymerase I
recombinant, *E. coli*

Cat. No.	Amount
EN-148S	300 Units
EN-148L	5 x 300 Units

Unit Definition: One unit is defined as the amount of enzyme required to convert 10 nmoles of dNTPs to an acid insoluble form in 30 minutes at 37 °C.

For *in vitro* use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Form: liquid (Supplied in 100 mM KPO₄ pH 6.5, 1 mM DTT and 50 % [v/v] glycerol)

Concentration: 5 units/μl

Applications:

- Fill-in of 5' overhangs to form blunt ends
- Removal of 3' overhangs to form blunt ends

Description:

Klenow Fragment is the large fragment of DNA Polymerase I that retains its 5'→3' polymerase, 3'→5' exonuclease and strand displacement activities. The enzyme lacks the 5'→3' exonuclease activity of intact DNA polymerase I. Klenow retains the polymerization fidelity of the holoenzyme without degrading 5' termini.

Reaction conditions:

- Dissolve 0.1 - 4 μg of digested DNA in 1x Reaction Buffer supplemented with 40 μM each dNTP
- Add 1 unit Klenow Fragment per μg DNA

Incubate for 15 min. at 25 °C

Stop reaction by alternatively

- add EDTA to 10 mM final concentration
- Heat inactivation: 20 min. at 75 °C

10x Reaction Buffer:

500 mM Tris-HCl pH 7.6 at 25 °C, 50 mM MgCl₂ and 10 mM DTT.

Note:

Excessive amounts of enzyme or longer reaction times may result in recessed ends due to the 3'→5' exonuclease activity of the enzyme.

Quality Control:

The enzyme is greater than 98 % pure as indicated by SDS-polyacrylamide gel electrophoresis and contains no detected endonuclease activity. Incubation of 10 units of Klenow with supercoiled plasmid DNA produced no nicked molecules after 20 hours at 37 °C as determined by agarose gel electrophoresis analysis.