DNA Polymerase I Large Fragment (Klenow Fragment)

Description: The Klenow Fragment lacks the 5′ → 3′ exonuclease activity of intact DNA Polymerase I but retains the 5′ → 3′ polymerase, the 3′ → 5′ exonuclease and the strand displacement activities.

Catalogue No	*201-1, 400 U* *201-2, 3x400 U*
Concentration	*5u/µl*

Reagents supplied: 10x Klenow Reaction buffer

Source: Purified from an *E. coli* strain carrying a DNA Polymerase I large fragment overproducing plasmid.

1X Klenow Reaction Buffer:

Reaction conditions: 50 mM Tris-HCl (pH 7.6 @ 25°C), 5 mM $MgCl_2$, 1 mM DTT and dNTPs. Klenow fragment is also 50% active in all five standard MINOTECH buffers when supplemented with dNTPs.

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.

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

Storage buffer: 0.1 M KPO_4 (pH 6.5), 1 mM DTT and 50% glycerol. Store at -20°C.

Heat inactivation: 75°C for 20 minutes.

Fill-in conditions: Dissolve 0.1-4 µg of digested DNA in 1x Klenow reaction buffer supplemented with 40 µM each dNTP. Add 1 unit Klenow per µg DNA and incubate 15 minutes at 25°C. Stop the reaction by adding EDTA to 10 mM final concentration and heating at 75°C for 10 minutes.

Note: excessive amounts of enzyme or longer reaction times may result in recessed ends due to the 3′ → 5′ exonuclease activity of the enzyme.