Data Sheet (30.05.2014)

DNA Polymerase I

Large Fragment (Klenow Fragment)

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

Cat.-No.	Size	Conc.
mi-E0148S	300 units	5 u/µl
mi-E0148L	1500 units	5 u/µl

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.

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.

Reaction conditions: 50 mM Tris-HCl (pH 7.6 @ 25 °C), 5 mM $MgCl_2$, 1 mM DTT and dNTPs (not included). Klenow fragment is also 50% active in all five metabion standard restriction enzyme buffers when supplemented with dNTPs.

Storage buffer: 0.1 M KPO_4 (pH 6.5), 1 mM DTT and 50 % glycerol.
Store at –20 °C. Avoid warming to 0 °C or higher.
Under these storage conditions, a guarantee of 12 months after delivery is given.

Heat inactivation: 75 °C for 20 minutes.

Fill-in conditions: Dissolve 0.1 – 4 µg of digested DNA in 1x 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.

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