

Product Insert

Klenow Fragment

Research Use Only

Product:

DNA Polymerase I, Klenow Fragment (Seq. grade)

Deoxynucleoside triphosphate:

DNA deoxynucleotidyltransferase E.C..2.7.7.7

Catalogue No.:

BIO-27029 500u

Description:

Klenow fragment is 68kDa proteolytic subfragement of *E. coli* DNA polymerase I, obtained by subtilising cleavage of the holenzyme. The enzyme is purified from *E. coli* PVG-AI strain.

Storage and dilution buffer:

50mM Tris-HCl, pH 7.5, 1mM dithiothreitol, 0.1mM EDTA and 50% glycerol.

Reaction buffer:

(10x): 100mM Tris-HCl (pH 7.5 at 25°C), 70mM ${\rm MgCl}_2$, 50mM DTT.

Batch details:

Batch No: See vial Units per vial: See vial Concentration: See vial

Suggestions for use:

Used in many molecular biology techniques. For further details, see Sambrook *et al* (1989) Molecular Cloning – A Laboratory Manual. Cold Spring Harbour Laboratory Press.

Storage conditions:

Klenow fragment can be stored at -20°C in a constant temperature freezer for 12 months. The enzyme will remain stable if stored as specified.

Unit definition:

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 37°C under standard assay conditions, using poly[d(A-T)] as template-primer.

Purity and associated activities:

Each preparation of Klenow fragment is checked for homogeneity on an SDS-polyacrylamide gel and is typically >95% pure as measured by densitometric scanning of the gel. The presence of detectable uncleaved DNA polymerase I is typically less than 1%. Endonuclease and exonuclease activities were not detectable after a 1 hour incubation of 1 µg of native lambda DNA and 0.22 µg of *Eco*RI digested lambda DNA, respectively at 37°C in the presence of 15-20 units of Klenow fragment. Each batch is functionally tested in Sanger sequencing protocols and fill-in reactions.

This product contains a declaration of analysis at the time of manufacture