Intact Genomics® Klenow exo-fragment

Catalog #	3264	3265
Package Size	200 units	1,000 units
Concentration	5 units/µl	

Description

Klenow exo- Fragment (3'→5' exo-) is a product of E. coli DNA Polymerase I which lacks the N-terminal 324 amino acids. This enzyme lacks the 5'→ 3' exonuclease activity of intact DNA Polymerase I, and has mutations (D355A, E357A) which abolish the 3´→ 5´ exonuclease activity but does exhibit the 5'→ 3' DNA polymerase activity (1). These characteristics make the enzyme useful for several molecular biology applications such as:

- Recombinase polymerase amplification (RPA) assay
- Di-deoxy sequencing (2)
- Second strand cDNA synthesis
- Second strand synthesis in mutagenesis (3)
- Single-stranded DNA probes generation

Protein Purity

The physical purity of this enzyme is ≥99% as assessed by SDS-PAGE with Coomassie® blue staining (see figure below).

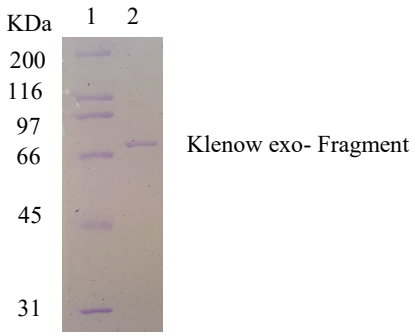

Fig: Lane 1, Protein marker
Lanes 2, Klenow exo- Fragment.

Product Includes

Klenow exo- Fragment
10x Klenow exo- reaction buffer

Storage temperature

–20°C

Storage Buffer

50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM β-mercaptoethanol, 1 mM DTT and 50% (v/v) glycerol.

Unit Definition

One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 37°C under standard assay conditions.

10x Reaction Buffer

500 mM Tris-HCl, 100 mM MgCl2, 50 mM dithiothreitol (DTT), pH 7.5 @ 25°C.

Product Source

E. coli cells carrying E. coli polA gene without its N-terminal exonuclease domain and carry D355A/E357A mutations.

Inactivation

Inactivated by heating at 75°C for 20 min.

Quality Control Assays

DNA Polymerase I, Klenow exo- Fragment is free from detectable endonuclease and RNase activities.

References

1. Derbyshire, V. et al. (1988). Science. 240, 199-201.
2. Sanger, F. et al. (1977). Proc. Natl. Acad. Sci. USA. 74, 5463-5467.
3. Gubler, U. (1987). In S.L. Berger & A.R. Rimmel (Ed.), Methods in Enzymology. 152, 330-335. San Diego: Academic Press.