

Klenow Fragment (exo⁻) (*Escherichia coli*)

Cat. No.	size
E1092-01	200 units
E1092-02	1000 units

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 10 nmoles of total nucleotides into acid-insoluble material in 30 minutes at 37°C.

Storage Conditions: Store at -20°C.

References:

1. Derbyshire, V., Freemont, P.S., Sanderson, M.R., Beese, L., Friedman, J.M., Joyce, C.M. and Steitz, T.A. (1988) *Science* 240, 199-201.
2. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74, 5463-5467
3. Houdebine, L. M. (1976) *Nucleic Acids Res.* 3, 615-630.
4. Sambrook, J., Fritsch, E.F., and Maniatis, T (1989) *Molecular Cloning: A Laboratory Manual*, second edition pp. 5.34, 5.40-5.43 Cold Spring Harbor Laboratory, Cold Spring Harbor.

Large fragment of *E. coli* DNA Polymerase I, which lacks both the 3'→5' exonuclease and the 5'→3' exonuclease activities of Polymerase I.

Description:

- Devoided of both the 3'→5' exonuclease and the 5'→3' exonuclease activities (1).
- Ultrapure recombinant enzyme.
- Used for Sanger dideoxy sequencing (2).
- Suitable for second strand cDNA synthesis (3).
- Used for 3'-end labeling and filling in 5'-protruding sticky ends (4).

Storage Buffer:

50 mM potassium phosphate (pH 7.0), 0.25 mM dithiothreitol and 50% (v/v) glycerol.

10 x Reaction Buffer:

500 mM Tris-HCl (pH 7.8 at 25°C), 100 mM MgSO₄, 10 mM dithiothreitol.

Assay Conditions:

67 mM potassium phosphate (pH 7.4), 6.7 mM MgCl₂, 1 mM dithiothreitol, 0.033 mM each dCTP, dGTP, dTTP and [α-³²P]dATP and 4.5 μg activated DNA. Incubation is at 37°C for 30 min in a reaction volume of 100 μl.

Quality Control:

All preparations are assayed for contaminating 3'- and 5'-exonuclease and endonuclease activities. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis.