Klenow Fragment

(Escherichia coli)

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Cat. No.	Size
E1091-01	200 units
E1091-02	1 000 units

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

Storage Conditions:
Store at -20˚C

Large fragment of *E. coli* DNA Polymerase I enzyme which retains both the polymerase and the proofreading 3'→5' exonuclease activities of Polymerase I.

Description:

- → Lacks the 5'-exonuclease activity (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.

Assay Conditions:

67 mM potassium phosphate (pH 7.4), 6.7 mM $MgCl_2$, 1 mM dithiothreitol, 0.033 mM each of dCTP, dGTP, dTTP and [α-^{32}P]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 endonuclease and 5'-exonuclease activities. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis.

References:

1. *Joyce, C.M. and Grindley, N.D. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 1830-1834.*
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.*
5. *Richardson, C.C., Schildkraut, C.L., Aposhian, H.V. and Kornberg, A. (1964) J. Biol. Chem. 239, 222-232.*