Invitrogen
life technologies

**Large Fragment of DNA Polymerase I**

**Cat. No. 18012-039** **Size: 500 units**
**Conc.: 3-9 U/µl** **Store at -20°C (not frost-free).**

Description:
Large Fragment of DNA Polymerase I (Klenow Fragment) is purified from *E. coli* expressing the Klenow fragment on a plasmid. This enzyme lacks the 5'→ 3' exonuclease activity of intact DNA Polymerase I, but does exhibit the 5'→ 3' DNA polymerase and 3'→ 5' exonuclease activities. Klenow Fragment is used primarily in dideoxy sequencing reactions (1,2), fill-in of restriction endonuclease termini (3), and second-strand cDNA synthesis (4).

Components:

| | |
|---|---|
| 18012-039 | Large Fragment of DNA Polymerase I |
| Y92500 | REact® 2 Buffer |
| 51669 | Klenow Dilution Buffer |

Unit Definition:
One unit incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 min at 37°C.

Klenow Dilution Buffer:
50 mM potassium phosphate (pH 7.0)
100 mM KCl
1 mM Dithiothreitol
50% (v/v) glycerol

10X REact® 2 Buffer:
500 mM Tris-HCl (pH 8.0)
100 mM $MgCl_2$
500 mM NaCl

Store REact® 2 Buffer and Klenow Dilution Buffer at -20°C.

Quality Control:
This product has passed the following quality control assays: SDS polyacrylamide gel analysis for purity; absence of detectable endodeoxyribonuclease, and self-priming activities; performance in a fill-in reaction.

Doc. Rev.: 092001

Fill-in Reaction Conditions:

1. Dilute Large Fragment of DNA Polymerase I to 0.5 U/µl with Klenow Dilution Buffer.
2. To a 1.5-ml microcentrifuge tube on ice, add:
   10X REact® 2 Buffer.....3 µl
   0.5 mM dATP .....1 µl
   0.5 mM dCTP .....1 µl
   0.5 mM dGTP .....1 µl
   0.5 mM dTTP.....1 µl
   DNA.....0.5-1 µg
   Large fragment of DNA Polymerase I.....1 µl
   Autoclaved distilled water.....to 30 µl
3. Mix gently and centrifuge briefly to bring the contents to the bottom of the tube.
4. Incubate at room temperature for 10-15 minutes or 20 minutes on ice.
5. Terminate fill-in reaction by phenol extraction.

To label the DNA fragment, use 1-2 µl of [$\alpha$-$^{32}$P]dNTP (400 Ci/mmol, 10 mCi/ml) (24-48 pmoles) instead of the corresponding cold dNTP.

References:

1. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. U.S.A.*, 15463.
2. Hartman, C.P. and Rabussay, D. (1981) in *Gene Amplification and Analysis* (Chirikjian, J.G., and Papas, T.S., eds.) Vol. 2, p. 17, Elsevier/North Holland, New York.
3. (1984) *Focus® 6:1*, 6.
4. Wickens, M.P. (1978) *J. Biol. Chem.* 253, 2483.