



## Large Fragment of DNA Polymerase I

**Cat. No. 18012-021**

**Size: 100 units**

**Conc.: 3-9 U/ $\mu$ l**

**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:

18012-021 Large Fragment of DNA Polymerase I  
Y92500 REAct<sup>®</sup> 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<sup>®</sup> 2 Buffer:

500 mM Tris-HCl (pH 8.0)  
100 mM MgCl<sub>2</sub>  
500 mM NaCl

Store REAct<sup>®</sup> 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.

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This product is distributed for laboratory research only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Invitrogen Tech-LineSM U.S.A. 800 955 6288

Fill-in Reaction Conditions:

1. Dilute Large Fragment of DNA Polymerase I to 0.5 U/ $\mu$ l with Klenow Dilution Buffer.
2. To a 1.5-ml microcentrifuge tube on ice, add:
 

10X REact <sup>®</sup> 2 Buffer.....	3 $\mu$ l
0.5 mM dATP .....	1 $\mu$ l
0.5 mM dCTP .....	1 $\mu$ l
0.5 mM dGTP .....	1 $\mu$ l
0.5 mM dTTP.....	1 $\mu$ l
DNA.....	0.5-1 $\mu$ g
Large fragment of DNA Polymerase I.....	1 $\mu$ l
Autoclaved distilled water.....	to 30 $\mu$ l
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  $\mu$ l of [ $\alpha$ -<sup>32</sup>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*<sup>®</sup> 6:1, 6.
4. Wickens, M.P. (1978) *J. Biol. Chem.* 253, 2483.