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: 10X REact® 2 Buffer:
50 mM potassium phosphate (pH 7.0) 500 mM Tris-HCl (pH 8.0)
100 mM KCl 100 mM MgCl₂
1 mM Dithiothreitol 500 mM NaCl
50% (v/v) glycerol

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 [α-32P]dNTP (400 Ci/mmol, 10 mCi/ml) (24-48 pmol) instead of the corresponding cold dNTP.

References: