DNA Polymerase I Large (Klenow) Fragment

CATALOG NO.: M1212-100

AMOUNT: 500 U (100 µl)

PRODUCT SOURCE: Recombinant *E. coli*

FORM: Liquid. Enzyme supplied with 10X Reaction Buffer

COMPONENTS:

Components Name	Volume	Part No.
DNA Polymerase I Large (Klenow) Fragment (5 U/µl)	100 µl	M1212-100-1
10X Large Klenow Fragment Reaction Buffer	300 µl	M1212-100-2

DESCRIPTION: DNA Polymerase I Large (Klenow) Fragment is the large fragment of E. coli DNA Polymerase I. The Klenow Fragment retains the DNA-dependent DNA polymerase activity of the E. coli DNA Polymerase I but lacks the 5'→3' exonuclease activity. With its inherent 3'→5' exonuclease activity, Klenow possesses the polymerization fidelity of the holoenzyme without degrading 5'-termini.

APPLICATIONS:

1. DNA blunting by filling-in 5'-overhangs with unlabeled or labeled dNTPs
2. cDNA second-strand synthesis
3. Generate single-stranded DNA probes using random primers
4. Site-directed DNA mutagenesis using synthetic oligonucleotides
5. Dideoxy DNA sequencing of single- or double-stranded DNA templates
6. 3'→5' exonuclease activity can blunt a 3'-overhang

STORAGE CONDITIONS: Store all components at -20°C. Avoid repeated freeze-thaw cycles of all components to retain maximum performance. All components are stable for one year from the date of shipping when stored and handled properly.

ENZYME STORAGE BUFFER: 25 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, and 50% (v/v) Glycerol.

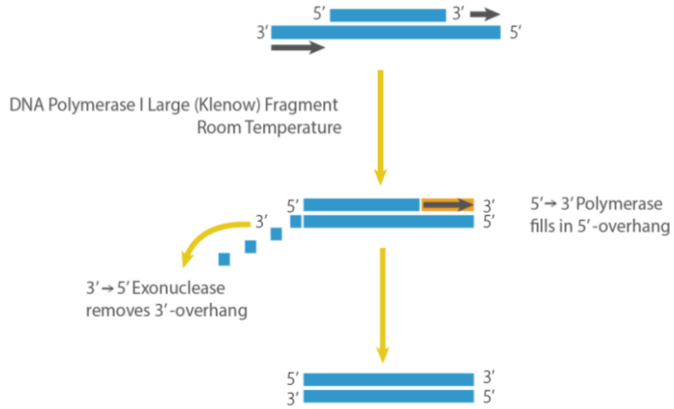
ENZYME UNIT DEFINITION: One unit is defined as the amount of DNA Polymerase I Large (Klenow) Fragment that catalyzes the incorporation of 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C using poly(dA-dT):poly(dA-dT) as a template:primer.

10X LARGE KLENOW FRAGMENT REACTION BUFFER: 100 mM Tris-HCl, 500 mM NaCl, 100 mM $MgCl_2$, 10 mM DTT, pH 7.9

REACTION CONDITIONS: Use 1X DNA Polymerase I Large (Klenow) Fragment Reaction Buffer and incubate at 37°C for 30 minutes.

Notes: To generate blunt ends by 3'-overhang removal and 3' recessed end fillin, DNA should be dissolved in 1X DNA Polymerase I Large (Klenow) Fragment Reaction Buffer supplemented with 33 µM of all four dNTPs. Use 1 unit of DNA Polymerase I Large (Klenow) Fragment per 1 µg DNA and incubate the reaction for 15 minutes at 25°C. Stop the reaction by adding 10 mM EDTA (final concentration) and heating at 75°C for 20 minutes.

HEAT INACTIVATION: 75°C for 20 minutes.



RELATED PRODUCTS:

- Advance™ DNA Polymerase (Cat# M1151-250, -1000)
- Blood Advance™ DNA Polymerase (Cat# M1153-100, -400)
- Breeze™ DNA Polymerase (Cat# M1148-250, -1000)
- Distant™ DNA Polymerase (Cat# M1150-250, -1000)
- Fire Start™ DNA Polymerase (Cat# M1149-250, -1000)
- Outstretched™ DNA Polymerase (Cat# M1152-250, -1000)
- PFU DNA Polymerase (Cat# 9003-500, -2500)
- Ready™ DNA Polymerase (Cat# M1146-1000, -5000, -10000)
- Robust Ready™ DNA Polymerase (Cat# M1147-250, -1000)
- Taq DNA Polymerase (Cat# 9001-500, -2500)

FOR RESEARCH USE ONLY! Not to be used on humans.

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