DNA Polymerase I, Large (Klenow) Fragment

WEB: www.abclonal.com

Catalog: RK20525
Size: 200 U / 1,000 U / 5,000 U
Concentration: 5,000 U/ml
Components:

DNA Polymerase I, Large (Klenow) Fragment (5,000 U/ml)	RM20515
10X ABuffer B	RM20126

Heat Inactivation: 75 ℃ for 20 min
Molecular Weight: Theoretical 68000 daltons
5' - 3' Exonuclease: No
3' - 5' Exonuclease: Yes
Strand Displacement: +
Error Rate: ~ $18x10^{-6}$ bases

Product Description

DNA Polymerase I, Large (Klenow) Fragment (about 68 kD) is a proteolytic product of *E.coli* DNA Polymerase I which retains polymerization and 3'→5' exonuclease activity, but has lost 5'→3' exonuclease activity. Klenow retains the polymerization fidelity of the holoenzyme without degrading 5' termini.
It is applicable to DNA sequencing by the Sanger dideoxy method, fill-in of 5´ overhangs to form blunt ends, removal of 3´ overhangs to form blunt ends, second strand cDNA synthesis and second strand synthesis in mutagenesis protocols.

Product Source:
An *E.coli* strain that contains the *E.coli* polA gene that has had its 5'→3' exonuclease domain removed.

Unit Definition:
One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37 ℃.

Storage Conditions:
25 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.4 @25 ℃

Storage Temperature: -20 ℃
Reaction Conditions: 1X ABuffer B, Incubate at 25 ℃

1X ABuffer B:
10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, pH7.9 @ 25 ℃

Instructions

Protocol for blunting ends by 3' overhang removal and fill-in of 3' recessed (5' overhang) ends using DNA Polymerase I, Large (Klenow) Fragment

1. DNA should be dissolved in 1X ABuffer A/B/C/S or T4 DNA Ligase Reaction buffer supplemented with 33 μM each dNTP.
2. Add 1 unit of Klenow per microgram DNA.
3. Incubate for 15 minutes at 25 ℃.
4. Stop reaction by adding EDTA to a final concentration of 10 mM and heating for 20 minutes at 75 ℃.

Notes:

- **CAUTION:** Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times may result in recessed ends due to the 3'→ 5' exonuclease activity of the enzyme.
- When DNA Polymerase I, Large (Klenow) Fragment is used to sequence DNA using the dideoxy method of Sanger *et al*., 1 unit/5 μl reaction volume is recommended.
- DNA Polymerase I, Large (Klenow) Fragment is also active in 1X ABuffer A/B/C/S and T4 DNA Ligase Reaction Buffer when supplemented with dNTPs.

QC Process:

- Purity is above 95% detected by SDS-PAGE.
- No nuclease, RNase contamination.
- No residual host genomic DNA is detected by PCR.