DNA Polymerase I, Large (Klenow) Fragment

Cat. No. : RK20525

Product components

Components	Component number	Concentration	200 U	1,000 U	5,000 U
DNA Polymerase I, Large (Klenow) Fragment	RM20515	5,000 U/mL	40 μL	200 μL	1 mL
10X ABuffer B	RM20126	10X	1.25 mL	1.25 mL	1.25 mL × 4

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.

Storage

-20℃

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℃.

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, pH 7.9 @ 25℃

Storage Conditions

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

Heat Inactivation

75℃ for 20 min

Strand Displacement

+

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Error Rate

~ $18x10^{-6}$ bases

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°C.
4. Stop reaction by adding EDTA to a final concentration of 10 mM and heating for 20 minutes at 75°C.

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.

1. 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.
2. 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.

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