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Klenow Fragment

Catalog Number: E111-1, E111-2

Table 1. Kit Components and Storage

Kit Component	E111-1 (200 units)	E111-2 (1000 units)	Storage	Stability
Klenow Fragment (5 units/µL)	40 µL	200 µL	-20 °C, avoid repeated free-thaw	The product is stable for 12 months when stored as directed.
10× Reaction Buffer	250 µL	1.25 mL		

Product Description

Klenow Fragment 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 Fragment retains the polymerization fidelity of the holoenzyme without degrading 5' termini.

The enzyme is available in 200 and 1000 unit sizes at a concentration of 5 U/µL. The enzyme is supplied with a 10× Reaction Buffer.

Applications

- Fill-in of 5´ overhangs to form blunt ends.
- Removal of 3´ overhangs to form blunt ends.
- DNA sequencing by the Sanger dideoxy method.
- Second strand cDNA synthesis.
- Second strand synthesis in mutagenesis protocols.

Product Specifications

- **Storage Buffer:** 25 mM Tris-HCl (pH 7.4 at 25 °C), 1 mM DTT, 0.1 mM EDTA, and 50% (v/v) glycerol.
- **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°C.
- **Unit Assay Conditions:** 1X Reaction Buffer, 33 µM dNTPs including [^{3}H]-dTTP and 70 µg/ml denatured herring sperm DNA.

Protocol for blunting ends by 3’ overhang removal and 5’ overhang end fill-in

1. Dissolve DNA sample in 1X reaction buffer supplemented with 33 µM dNTPs.
2. Add 1 unit Klenow Fragment per microgram DNA.
3. Incubate at 25 °C for 15 min.
4. Stop the reaction by heating at 75 °C for 20 min or adding EDTA to a final concentration of 10 mM.