

Klenow

Tested User Friendly



Fill-In Protocol

Klenow (PN 2141), the large fragment of DNA polymerase I from *E. coli*, is well suited for use in 3'-end labeling of DNA fragments for sequence analysis, for the conversion of 5'-extended ends to flush ends for cloning, for second-strand synthesis of cDNA and for random primed labeling of DNA fragments. It lacks the 5' to 3' exonuclease activity of intact DNA polymerase I.

Product Notes:

Molecular Weight: 68,000

Optimum pH: 7.4

Optimum Temperature: 37°C

Requirement for Divalent Cation: Mg²⁺, Mn²⁺

Optimum Mg²⁺ Concentration: 7mM

Klenow and 10X Klenow Reaction Buffer have been functionally tested in the following protocol:

Fill-in Labeling Protocol:

10X Klenow Reaction Buffer (included)

0.5M Tris-HCl, pH 7.5

0.1M MgCl₂

10mM DTT

0.5 mg/ml BSA (nuclease-free)

For a 20μl reaction:

0.1 to 4μg	DNA with 5' overhanging ends
2μl	Radiolabeled-dATP (400 to 800 Ci/mmol)
1μl	0.5mM each dGTP, dCTP, dTTP
2μl	10X Klenow Reaction Buffer
1-5 units	Klenow
to 20μl	H ₂ O

Incubate for 15 minutes at room temperature. Stop the reaction with 1μl of 0.5M EDTA or by heating at 75°C for 10 minutes. Remove unincorporated dNTP precursors from labeled DNA, if desired.

Repairing 3' Recessed Ends to Generate Blunt Ends

For a 20μl reaction:

0.1 to 4μg	Restricted DNA
1μl	0.5mM each dNTP
2μl	10X Klenow Reaction Buffer
1-5 units	Klenow
to 20μl	H ₂ O

Incubate for 15 minutes at room temperature. Stop the reaction by heating to 75°C for 10 minutes or by adding 1μl 0.5M EDTA.

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