

Klenow Fragment of DNA Polymerase I

Catalogue number: MB00901, 300 U

Description

Klenow Fragment of DNA Polymerase I is a truncated fragment of *E. coli* DNA polymerase I that lacks the 5' → 3' exonuclease activity while retaining the 3' → 5' exonuclease activity (proofreading). The enzyme is well suited for use in 3'-end labelling of DNA fragments for sequence analysis, for the conversion of 5'-overhangs to create blunt-ends for cloning, for the removal of 3'-overhangs, for second-strand synthesis of cDNA and for random primer labelling of DNA fragments.

Storage Buffer

20 mM Tris-HCl, pH 7.5, 1.0 mM DTT, 0.1 mM EDTA, 50% glycerol. Store at -20 °C.

Concentration: 5 U/μL

Unit definition

One unit incorporates 10 nmol of total nucleotides into an acid-insoluble material in 30 min at 37 °C.

Reaction buffer (10×): 500 mM Tris-HCl, pH 7.5, 100 mM MgCl₂, 10 mM DTT.

Related products:

Product name	Cat. No.
Alkaline Phosphatase	MB018
T4 Polynucleotide Kinase	MB008
Speedy Ligase	MB130
NZYGelpure	MB011

Fill-in labelling protocol

- Combine the following components:

DNA with 5'-overhangs (0.1 to 4 μg)	__μL
Radiolabeled dNTP (400 to 800 Ci/mol)	1 μL
Non-labelled dNTP (2 mM each)	0.5 μL
10× Reaction buffer	2 μL
Water	__μL
Klenow Fragment of DNA Polymerase I	1-2 units
Final volume	20 μL

- Mix and incubate at 37 °C for 15 min.
- Terminate the reaction by heating at 75 °C for 10 minutes. Remove unincorporated dNTP precursors from labelled DNA, if desired.

Note: The enzyme incorporates modified nucleotides (e.g. biotin-, digoxigenin-, fluorescently-labeled nucleotides).

Fill-in protocol to generate blunt ends

- Combine the following components:

DNA with 5'-overhangs (0.1 to 4 μg)	__μL
dNTPs (2 mM each)	0.5 μL
10× Reaction buffer	2 μL
Water	__μL
Klenow Fragment of DNA Polymerase I	1-5 units
Final volume	20 μL

- Mix and incubate at 37 °C for 15 min.
- Terminate the reaction by heating at 75 °C for 10 minutes.

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases assays	Pass
Functional assay	Pass

Approved by:



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