

## Klenow Fragment (3'→5' exo<sup>-</sup>)



1-800-632-7799  
info@neb.com  
www.neb.com



# M0212S



**200 units**      **5,000 U/ml**      **Lot: 0351309**  
**RECOMBINANT**    **Store at -20°C**    **Exp: 9/15**

**Description:** Klenow Fragment (3'→5' exo<sup>-</sup>) is an N-terminal truncation of DNA Polymerase I which retains polymerase activity, but has lost the 5'→3' exonuclease activity and has mutations (D355A, E357A) which abolish the 3'→5' exonuclease activity (1).

**Source:** An *E. coli* strain containing a plasmid with a fragment of the *E. coli polA* (D355A, E357A) gene starting at codon 324.

### Applications:

- Random priming labeling
- DNA sequencing by the Sanger dideoxy method (2)
- Second strand cDNA synthesis
- Second strand synthesis in mutagenesis protocols (3).

Supplied in: 25 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

**Reagents Supplied with Enzyme:**  
10X NEBuffer 2.

**Reaction Conditions:** 1X NEBuffer 2.  
Supplement with dNTPs (not included).

Klenow Fragment (3'→5' exo<sup>-</sup>) is also active in all four NEBuffers when supplemented with dNTPs.

**1X NEBuffer 2:**  
50 mM NaCl  
10 mM Tris-HCl  
10 mM MgCl<sub>2</sub>  
1 mM DTT  
pH 7.9 @ 25°C

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**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 NEBuffer 2, 33 μM dNTPs including [<sup>3</sup>H]-dTTP and 70 μg/ml denatured herring sperm DNA.

**DNA Sequencing:** When this preparation is used to sequence DNA using the dideoxy method of Sanger et al. 1 unit/5 μl reaction volume is recommended.

**Molecular Weight:** 68,000 daltons.

**Heat Inactivation:** 75°C for 20 minutes.

### Quality Control Assays

**Exonuclease Activity:** Incubation of a 50 μl reaction in NEBuffer 2 containing a minimum of 200 units of Klenow Fragment (3'→5' exo<sup>-</sup>) with 1 μg of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA for 4 hours at 37°C releases < 0.1% of the total radioactivity.

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**3'→5' Exonuclease Activity:** Incubation of a 20 μl reaction in NEBuffer 2 containing a minimum of 50 units of Klenow Fragment (3'→5' exo<sup>-</sup>) with 10 nM fluorescent internally labeled oligonucleotide for 30 minutes at 37°C yields no detectable 3'→5' degradation as determined by capillary electrophoresis.

**Endonuclease Activity:** Incubation of a 50 μl reaction in NEBuffer 2 containing a minimum of 50 units of Klenow Fragment (3'→5' exo<sup>-</sup>) with 1 μg of supercoiled φX174 DNA for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Physical Purity:** Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

**Notes On Use:** Klenow Fragment (3'→5' exo<sup>-</sup>) is not suitable for generating blunt ends because it lacks the 3'→5' exonuclease necessary to remove non-templated 3' additions.

(see other side)

CERTIFICATE OF ANALYSIS

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**References:**

1. Derbyshire, V. et al. (1988) *Science* 240, 199–201.
2. Sanger, F. et al. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463–5467.
3. Gubler, U. (1987). In S.L. Berger and A.R. Kimmel (Eds.), *Methods in Enzymology* Vol.152, (pp. 330–335). San Diego: Academic Press.

**Companion Products Sold Separately:**

NEBuffer 2  
#B7002S 6.0 ml

Deoxynucleotide Solution Set  
#N0446S 25 µmol of each

Deoxynucleotide Solution Mix  
#N0447S 8 µmol each  
#N0447L 40 µmol each

**References:**

1. Derbyshire, V. et al. (1988) *Science* 240, 199–201.
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