Klenow Fragment (3´→5´ exo⁻)

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

M0212S 035120914091

M0212S

200 units **5,000 U/ml** **Lot: 0351209**
RECOMBINANT **Store at –20°C** **Exp: 9/14**

Description: Klenow Fragment (3´→ 5´ exo⁻) 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⁻) is also active in all four NEBuffers when supplemented with dNTPs.

1X NEBuffer 2:
50 mM NaCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.9 @ 25°C

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 [^{3}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⁻) with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA for 4 hours at 37°C releases < 0.1% of the total radioactivity.

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⁻) 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⁻) 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⁻) 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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Page 2 (M0212)