Klenow Fragment $(3'\rightarrow 5' \text{ exo}^-)$





M0212S



200 units 5.000 U/ml Lot: 0351209 RECOMBINANT Store at -20°C Exp: 9/14

Description: Klenow Fragment $(3' \rightarrow 5' \text{ exo}^-)$ is an N-terminal truncation of DNA Polymerase I which retains polymerase activity, but has lost the $5' \rightarrow 3'$ exonuclease activity and has mutations (D355A, E357A) which abolish the $3' \rightarrow 5'$ 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-HCI (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' \rightarrow 5' exo⁻) is also active in all four NEBuffers when supplemented with dNTPs.

1X NEBuffer 2:

50 mM NaCl 10 mM Tris-HCI 10 mM MgCl_o 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 [3H]-dTTP and 70 ug/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 ul reaction in NEBuffer 2 containing a minimum of 200 units of Klenow Fragment (3 \rightarrow 5' exo-) with 1 µg of a mixture of single and double-stranded [3H] 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 \rightarrow 5 $^{\prime}$ exo-) with 10 nM fluorescent internally labeled oligonucleotide for 30 minutes at 37°C yields no detectable $3 \rightarrow 5$ 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 \rightarrow 5' exo-) with 1 ug of supercoiled ϕ X174 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 $(3' \rightarrow 5' \text{ exo}^-)$ is not suitable for generating blunt ends because it lacks the $3 \rightarrow 5$ exonuclease necessary to remove non-templated 3' additions.

(see other side)

CERTIFICATE OF ANALYSIS

Klenow Fragment $(3'\rightarrow 5' \text{ exo}^-)$

M0212S

200 units



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

RN NEB 2 Yes

Lot: 0351209

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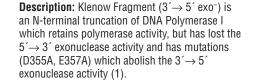
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sperm DNA.

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 μ mol of each

Deoxynucleotide Solution Mix

#N0447S 8 µmol each

#N0447L 40 µmol each

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References:

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