Exonuclease I (*E. coli*)

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

M0293S 024160418041

M0293S

RR 37° Yes

3,000 units 20,000 U/ml Lot: 0241604

RECOMBINANT Store at –20°C Exp: 4/18

Description: Catalyzes the removal of nucleotides from single-stranded DNA in the 3´ to 5´ direction.

Source: An *E. coli* strain that carries the cloned *Exo* I gene from *E. coli* NM554

Applications:

- Exonuclease I degrades excess single-stranded primer oligonucleotide from a reaction mixture containing double-stranded extension products.

Storage Conditions: 100 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 5 mM 2-mercaptoethanol, 100 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X Exonuclease I Reaction Buffer

Reaction Conditions: 1X Exonuclease I Reaction Buffer. Incubate at 37°C.

1X Exonuclease I Reaction Buffer:
67 mM Glycine-KOH
6.7 mM $MgCl_2$
10 mM 2-mercaptoethanol
(pH 9.5 @ 25°C)

Unit Definition: One unit is defined as the amount of enzyme that will catalyze the release of 10 nmol of acid-soluble nucleotide in 30 minutes at 37°C under standard reaction conditions.

Unit Assay Conditions: 67 mM Glycine-KOH (pH 9.5), 6.7 mM $MgCl_2$, 10 mM 2-mercaptoethanol, and 0.17 mg/ml single-stranded [^{3}H]-DNA.

Heat Inactivation: 80°C for 20 minutes.

Quality Control Assays

Double-stranded Endonuclease: Incubation of 100 units of Exonuclease I with 1 µg ϕX174 RF I DNA for 16 hours at 37°C in 50 µl reaction buffer resulted in < 5% conversion to RF II.

Single-stranded Endonuclease: Incubation of 100 units of Exonuclease I with 1 µg of M13mp18 single-stranded DNA for 16 hours at 37°C in a 50 µl reaction resulted in no decrease in the amount of closed circular DNA as determined by agarose gel electrophoresis.

Double-stranded Exonuclease: Incubation of 50 units of enzyme with 0.2 µg ^{3}H DNA (9.0×10^3 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.5% radioactivity.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection. BSA is added to the enzyme for stability.

References:

1. Lehman and Nussbaum (1964) *J. Biol. Chem.* 239, 2628.
2. Kusher et al. (1971) *Proc. Natl. Acad. Sci. USA* 68, 824.
3. Kusher et al. (1972) *Proc. Natl. Acad. Sci. USA* 69, 1366.
4. Goldmark and Linn (1972) *J. Biol. Chem.* 247, 184.
5. Rosamond et al. (1979) *J. Biol. Chem.* 254, 8646.

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CERTIFICATE OF ANALYSIS

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