# Exonuclease I
# (*E. coli*)

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

## M0293S

**3,000 units** **20,000 U/ml** **Lot: 0211510**
**RECOMBINANT** **Store at –20°C** **Exp: 10/17**

**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 ($9.0 \times 10^3$ 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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