Exonuclease I  
*(E. coli)*

**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 MgCl₂
- 10 mM 2-mercaptoethanol

**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 MgCl₂, 10 mM 2-mercaptoethanol, and 0.17 mg/ml single-stranded [3H]-DNA.

**Heat Inactivation:** 80°C for 20 minutes.

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**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.

**Doublstranded Endonuclease:** Incubation of 50 units of enzyme with 0.2 µg [3H] DNA (9.0 x 10³ 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.

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References:


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