Exonuclease I (E. coli)

**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
- Reaction Buffer. Incubate at 37°C.

**Unit Assay Conditions:**
- 67 mM Glycine-KOH
- 6.7 mM MgCl₂
- 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.

**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.

**References:**

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