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

Heat Inactivation: 80°C for 20 minutes.

Quality Control Assays

Double-stranded Endonuclease: Incubation of 100 units of Exonuclease I with 1 µg ofX174 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 ofH DNA (9.0 x 10⁶ 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.