NEW ENGLAND Biolabs®

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New England Biolabs
Product Specification

Product Name:	*Exonuclease I (E. coli)*
Catalog #:	*M0293S/L/V*
Concentration:	*20,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme that will catalyze the release of 10 nmol of acid-soluble nucleotide in a total reaction volume of 100 µl in 30 minutes at 37°C in 1X Exonuclease I Reaction Buffer with 0.17 mg/ml single-stranded [^{3}H]-DNA.*
Shelf Life:	*24 months*
Storage Temp:	*-20°C*
Storage Conditions:	*100 mM NaCl, 10 mM Tris-HCl, 0.5 mM EDTA, 5 mM BME, 50 % Glycerol, 100 µg/ml BSA, (pH 7.5 @ 25°C)*
Specification Version:	*PS-M0293S/L v1.0*
Effective Date:	*11 Aug 2016*

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Circular Single Stranded DNA) - A 50 µl reaction in Exonuclease I Reaction Buffer containing 1 µg of M13mp18 Single-stranded DNA and a minimum of 100 units of Exonuclease I (*E. coli*) incubated for 16 hours at 37°C results in <10% conversion to linear DNA as determined by agarose gel electrophoresis.

Endonuclease Activity (Nicking) - A 50 µl reaction in Exonuclease I Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of Exonuclease I (*E. coli*) incubated for 16 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release, Double Stranded) - A 50 µl in Exonuclease I Reaction Buffer containing 0.2 µg [^{3}H] CpG methylated Lambda DNA and a minimum of 50 units of Exonuclease I (*E. coli*) incubated for 4 hours at 37°C releases <0.5% of the total radioactivity.

Protein Purity Assay (SDS-PAGE) - Exonuclease I (*E. coli*) is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 20 units of Exonuclease I (*E. coli*) is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.

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Assay Name/Specification (minimum release criteria)
RNase Activity (Extended Digestion) - A 10 µL reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of Exonuclease I (*E. coli*) is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Date 11 Aug 2016

Derek Robinson
Quality Approver