NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*Thermolabile Exonuclease I*
Catalog #:	*M0568S/L*
Concentration:	*20,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme that will catalyze the release of 2 nmol of acid-soluble nucleotide in a total reaction volume of 100 µl in 6 minutes at 37°C in NEBuffer 3.1 with 0.17 mg/ml single-stranded [^{3}H]-E.coli DNA.*
Shelf Life:	*12 months*
Storage Temp:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl, 250 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA, 50% Glycerol, (pH 7.4 @ 25°C)*
Specification Version:	*PS-M0568S/L v1.0*
Effective Date:	*06 Apr 2018*

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Circular Single Stranded DNA) - A 50 µl reaction in CutSmart® Buffer containing 1 µg of M13 single-stranded DNA and a minimum of 100 units of Thermolabile Exonuclease I incubated for 4 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 CutSmart® Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of Thermolabile Exonuclease I incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Functional Testing (Thermolability) - A 20 µl reaction in Standard *Taq* Reaction Buffer containing 20 pmol of 20-mer ssDNA and 20 units of Thermolabile Exonuclease I was incubated for 4 minutes at 37°C followed by heat inactivation for 1 minute at 80°C. The addition of 20 pmol of 20-mer ssDNA and incubation for 40 minutes at 37°C results in no cleavage of additional substrate as determined by capillary electrophoresis.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in CutSmart® Buffer containing 1 µg of PhiX174-HaeIII DNA and a minimum of 100 units of Thermolabile Exonuclease I incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

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

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Assay Name/Specification (minimum release criteria)
qPCR DNA Contamination (E. coli Genomic) - A minimum of 20 units of Thermolabile Exonuclease I 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.
RNase Activity Assay (4 Hour 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 Thermolabile Exonuclease I is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Date 06 Apr 2018

Derek Robinson
Director of Quality Control

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices