240 County Road
Ipswich, MA 01938-2723
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New England Biolabs
Certificate of Analysis

Product Name:	*Thermolabile Exonuclease I*
Catalog Number:	*M0568S*
Concentration:	*20,000 U/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 [³H]-E.coli DNA.*
Packaging Lot Number:	*10247567*
Expiration Date:	*05/2026*
Storage Temperature:	*-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*

Thermolabile Exonuclease I Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0568SVIAL	Thermolabile Exonuclease I	10240888	**Pass**
B6003SVIAL	NEBuffer™ r3.1	10237086	**Pass**

Assay Name/Specification	**Lot # 10247567**
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 37ºC results in <10% conversion to linear DNA as determined by agarose gel electrophoresis.	**Pass**
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.	**Pass**
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	**Pass**

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Assay Name/Specification	Lot # 10247567
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 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Protein Purity Assay (SDS-PAGE) Thermolabile Exonuclease I is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
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.	**Pass**
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.	**Pass**

This product has been tested and shown to be in compliance with all specifications.

Heidi Church
Production Scientist
24 May 2024

Michael Tonello
Packaging Quality Control Inspector
27 Jun 2024