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240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## 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:	10178087
Expiration Date:	08/2024
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				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
M0568SVIAL	Thermolabile Exonuclease I	10160873	Pass	
B6003SVIAL	NEBuffer™ r3.1	10168652	Pass	

Assay Name/Specification	Lot # 10178087
<b>RNase Activity Assay (4 Hour Digestion)</b> A 10 $\mu$ I reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ I 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
<b>Functional Testing (Thermolability)</b> 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.	Pass
<b>Endonuclease Activity (Circular Single Stranded DNA)</b> 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	Pass





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Assay Name/Specification	Lot # 10178087
electrophoresis.	
<b>qPCR DNA Contamination (E. coli Genomic)</b> 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 $\leq$ 1 E. coli genome.	Pass
Protein Purity Assay (SDS-PAGE) Thermolabile Exonuclease I is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>Endonuclease Activity (Nicking)</b> 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
<b>Non-Specific DNase Activity (16 Hour)</b> 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.	Pass

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

m J. Loei

John Greci Production Scientist 14 Sep 2022

Josh Hersey

Packaging Quality Control Inspector 07 Feb 2023

