

## New England Biolabs Certificate of Analysis

**Product Name:** *Thermolabile Exonuclease I*  
**Catalog Number:** M0568L  
**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 [<sup>3</sup>H]-E.coli DNA.  
**Packaging Lot Number:** 10254555  
**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
M0568LVIAL	Thermolabile Exonuclease I	10240889	Pass
B6003SVIAL	NEBuffer™ r3.1	10237086	Pass

Assay Name/Specification	Lot # 10254555
<p><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 &lt;10% conversion to linear DNA as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><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 &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><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</p>	<b>Pass</b>

Assay Name/Specification	Lot # 10254555
additional substrate as determined by capillary electrophoresis.	
<p><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.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> Thermolabile Exonuclease I is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>RNase Activity Assay (4 Hour Digestion)</b> 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, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	<b>Pass</b>
<p><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 ≤ 1 E. coli genome.</p>	<b>Pass</b>

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

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24 May 2024



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13 Aug 2024