

## New England Biolabs Certificate of Analysis

**Product Name:** *Thermolabile USER<sup>®</sup> II Enzyme*

**Catalog Number:** *M5508L*

**Concentration:** *1,000 U/ml*

**Unit Definition:** *One unit is defined as the amount of enzyme required to nick 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base in 15 minutes at 37°C in a total reaction volume of 10 µL in 1X T4 DNA Ligase Buffer.*

**Packaging Lot Number:** *10159631*

**Expiration Date:** *08/2024*

**Storage Temperature:** *-20°C*

**Storage Conditions:** *25 mM KCl, 35 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 15 mM Tris-HCl, 100 µg/ml BSA, 50 % Glycerol, (pH 7.5 @ 25°C)*

**Specification Version:** *PS-M5508S/L v1.0*

Thermolabile USER <sup>®</sup> II Enzyme Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M5508LVIAL	Thermolabile USER <sup>®</sup> II Enzyme	10159630	Pass
B6004SVIAL	rCutSmart <sup>™</sup> Buffer	10162782	Pass

Assay Name/Specification	Lot # 10159631
<p><b>RNase Activity (Extended 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 USER<sup>®</sup> II Enzyme is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	Pass
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 1 unit of Thermolabile USER<sup>®</sup> II Enzyme is screened for the presence of E. coli genomic DNA using SYBR<sup>®</sup> 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>	Pass
<p><b>Functional Testing (Thermolability, Endonuclease III)</b> A 10 µl reaction in CutSmart<sup>®</sup> Buffer containing 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base and 1 unit of Thermolabile USER<sup>®</sup> II Enzyme was incubated for 15 minutes at 37°C followed by heat</p>	Pass

Assay Name/Specification	Lot # 10159631
<p>inactivation for 10 minutes at 65°C. The addition of 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single AP site and incubation for 15 minutes at 37°C followed by 10 minutes at 75°C, results in no cleavage of additional substrate.</p>	
<p><b>Functional Testing (Thermolability, UDG)</b> A 10 µl reaction in CutSmart® Buffer containing 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base and 1 unit of Thermolabile USER® II Enzyme was incubated for 15 minutes at 37°C followed by heat inactivation for 10 minutes at 65°C. The addition of 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base with 20 units of Endonuclease III and incubation for 15 minutes at 37°C followed by 10 minutes at 75°C, results in no cleavage of additional substrate.</p>	<b>Pass</b>
<p><b>Functional Testing (USER, Transformation assay)</b> A 10 µl reaction in ThermoPol® Reaction Buffer containing 20 ng linearized pNEB206A, 100 ng of a 950 bp control PCR product and 1 unit of Thermolabile USER® II Enzyme was incubated for 15 minutes at 37°C followed by 15 minutes at 25°C. After transformation into ER2267 chemically-competent cells &gt;95% of colonies contained recombinant plasmid.</p>	<b>Pass</b>

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](http://www.neb.com/trademarks) for additional information.

*Lauren Higgins*

Lauren Sears Higgins  
Production Scientist  
28 Jul 2022

*Josh Hersey*

Josh Hersey  
Packaging Quality Control Inspector  
13 Oct 2022