NEW ENGLAND BioLabs Inc.
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New England Biolabs
Certificate of Analysis

Product Name:	*Thermolabile USER® 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:	*10091200*
Expiration Date:	*10/2022*
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® II Enzyme Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M5508LVIAL	Thermolabile USER® II Enzyme	10086075	**Pass**
B7204SVIAL	CutSmart® Buffer	10089400	**Pass**

Assay Name/Specification	**Lot # 10091200**
RNase Activity (Extended 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 USER® II Enzyme is incubated at 37ºC. After incubation for 16 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 1 unit of Thermolabile USER® II Enzyme 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**
Functional Testing (USER, Transformation assay) 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	**Pass**

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Assay Name/Specification	Lot # 10091200
transformation into ER2267 chemically-competent cells >95% of colonies contained recombinant plasmid.	
Functional Testing (Thermolability, UDG) 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 37ºC followed by heat inactivation for 10 minutes at 65ºC. 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 37ºC followed by 10 minutes at 75ºC, results in no cleavage of additional substrate.	**Pass**
Functional Testing (Thermolability, Endonuclease III) 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 37ºC followed by heat inactivation for 10 minutes at 65ºC. The addition of 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single AP site and incubation for 15 minutes at 37ºC followed by 10 minutes at 75ºC, results in no cleavage of additional substrate.	**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.

Lauren Higgins
Production Scientist
20 Nov 2020

Michael Tonello
Packaging Quality Control Inspector
20 Nov 2020