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New England Biolabs Certificate of Analysis

Product Name: Thermolabile USER® II Enzyme

Catalog Number: M5508S
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: 10101179
Expiration Date: 03/2023
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	
M5508SVIAL	Thermolabile USER® II Enzyme	10100386	Pass	
B7204SVIAL	CutSmart® Buffer	10096305	Pass	

Assay Name/Specification	Lot # 10101179
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
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 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	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 37°C followed by heat	Pass



M5508S / Lot: 10101179

Page 1 of 2

Assay Name/Specification	Lot # 10101179
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.	
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 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.	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 transformation into ER2267 chemically-competent cells >95% of colonies contained recombinant plasmid.	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

Lauren Higgins

11 Mar 2021

Michael Tonello

Packaging Quality Control Inspector

11 Mar 2021



M5508S / Lot: 10101179

Page 2 of 2