

## New England Biolabs Certificate of Analysis

**Product Name:** *Thermolabile USER<sup>®</sup> 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:** *10182213*

**Expiration Date:** *02/2025*

**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*

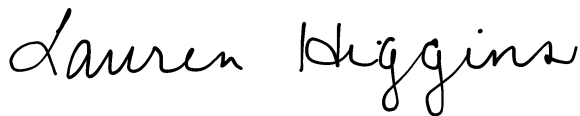
| Thermolabile USER <sup>®</sup> II Enzyme Component List |  |            |                      |
|---|--|------------|----------------------|
| NEB Part Number   | Component Description                    | Lot Number | Individual QC Result |
| M5508SVIAL  | Thermolabile USER <sup>®</sup> II Enzyme | 10178402   | Pass                 |
| B6004SVIAL  | rCutSmart <sup>™</sup> Buffer            | 10175292   | Pass                 |

| Assay Name/Specification   | Lot # 10182213 |
|--|----------------|
| <p><b>Functional Testing (USER, Transformation assay)</b><br/>A 10 µl reaction in ThermoPol<sup>®</sup> Reaction Buffer containing 20 ng linearized pNEB206A, 100 ng of a 950 bp control PCR product and 1 unit of Thermolabile USER<sup>®</sup> 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>   | Pass           |
| <p><b>Functional Testing (Thermolability, UDG)</b><br/>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 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> | Pass           |
| <p><b>Functional Testing (Thermolability, Endonuclease III)</b></p>  | Pass           |

| Assay Name/Specification   | Lot # 10182213 |
|--|----------------|
| <p>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 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>RNase Activity (Extended Digestion)</b><br/>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, &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><br/>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.</p>  | <b>Pass</b>    |

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

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