NEW ENGLAND BioLabs Inc.
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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:* | *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® II Enzyme Component List** | | | |
|---|---|---|---|
| **NEB Part Number** | **Component Description** | **Lot Number** | **Individual QC Result** |
| M5508SVIAL | Thermolabile USER® II Enzyme | 10178402 | **Pass** |
| B6004SVIAL | rCutSmart™ Buffer | 10175292 | **Pass** |

| **Assay Name/Specification** | **Lot # 10182213** |
|---|---|
| **Functional Testing (USER, Transformation assay)**<br>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** |
| **Functional Testing (Thermolability, UDG)**<br>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)** | **Pass** |

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| Assay Name/Specification | Lot # 10182213 |
|---|---|
| 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. | |
| **RNase Activity (Extended Digestion)**<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 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)**<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. | **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 Sears Higgins
Production Scientist
24 Feb 2023

Michael Tonello
Packaging Quality Control Inspector
27 Feb 2023