

## New England Biolabs Certificate of Analysis

**Product Name:** USER<sup>®</sup> Enzyme  
**Catalog Number:** M5505S  
**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 oligonucleotide duplex containing a single uracil base, in 15 minutes at 37°C in a total reaction volume of 10 µl.  
**Packaging Lot Number:** 10092240  
**Expiration Date:** 04/2022  
**Storage Temperature:** -20°C  
**Storage Conditions:** 50 mM KCl, 5 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 175 µg/ml BSA, (pH 7.4 @ 25°C)  
**Specification Version:** PS-M5505S/L v1.0

| USER <sup>®</sup> Enzyme Component List |                              |            |                      |
|-----------------------------------------|------------------------------|------------|----------------------|
| NEB Part Number                         | Component Description        | Lot Number | Individual QC Result |
| M5505SVIAL                              | USER <sup>®</sup> Enzyme     | 10071830   | Pass                 |
| B7204SVIAL                              | CutSmart <sup>®</sup> Buffer | 10091036   | Pass                 |

| Assay Name/Specification                                                                                                                                                                                                                                                                                                                                                                                                                   | Lot # 10092240 |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| <p><b>Functional Test (USER, Transformation assay)</b><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 USER<sup>®</sup> 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>qPCR DNA Contamination (E. coli Genomic)</b><br/>           A minimum of 1 unit of USER<sup>®</sup> 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>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 USER<sup>®</sup> 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           |

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*

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30 Dec 2020

*Michael Tonello*

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30 Dec 2020