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## New England Biolabs Certificate of Analysis

Product Name: USER® Enzyme
Catalog #: M5505S/L
Concentration: 1,000 units/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  $\mu$ l.

 Lot #:
 0161804

 Assay Date:
 04/2018

 Expiration Date:
 4/2020

 Storage Temp:
 -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

Effective Date: 07 Feb 2018

| Assay Name/Specification (minimum release criteria)  | Lot #0161804 |
|--|--------------|
| Functional Test (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 USER <sup>TM</sup> 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         |
| <b>qPCR DNA Contamination (</b> <i>E. coli</i> <b>Genomic)</b> - A minimum of 1 unit of USER <sup>TM</sup> Enzyme is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is $\leq 1$ <i>E. coli</i> 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 USER <sup>TM</sup> 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         |

<sup>\*</sup> The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

Authorized by Derek Robinson 07 Feb 2018







Inspected by
Lauren Higgins
19 Apr 2018