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## New England Biolabs Certificate of Analysis

Product Name: USER® Enzyme

Catalog Number: M5505L 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: 10182267
Expiration Date: 08/2024
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® Enzyme Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
M5505LVIAL	USER® Enzyme	10159205	Pass	
B6004SVIAL	rCutSmart™ Buffer	10178016	Pass	

Assay Name/Specification	Lot # 10182267
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® 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
RNase Activity (Extended Digestion) A 10 $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ l of USER® 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
<b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 1 unit of USER® 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



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

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Lauren Sears Higgins Production Scientist 22 Jul 2022 Michael Tonello
Packaging Quality Control Inspector

31 Mar 2023