

## 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:** 10229458  
**Expiration Date:** 12/2025  
**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			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M5505LVIAL	USER® Enzyme	10227675	Pass
B6004SVIAL	rCutSmart™ Buffer	10224841	Pass

Assay Name/Specification	Lot # 10229458
<p><b>Functional Test (USER, Transformation assay)</b>            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 &gt;95% of colonies contained recombinant plasmid.</p>	<b>Pass</b>
<p><b>RNase Activity (Extended Digestion)</b>            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® 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>            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.</p>	<b>Pass</b>

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

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08 Feb 2024



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Josh Hersey  
Packaging Quality Control Inspector  
12 Feb 2024