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New England Biolabs
Product Specification

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 µl.*
Shelf Life:	*24 months*
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 rAlbumin (pH 7.4 @ 25°C)*
Specification Version:	*PS-M5505S/L v2.0*
Effective Date:	*18 Mar 2024*

Assay Name/Specification (minimum release criteria)
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.
qPCR DNA Contamination (*E. coli* Genomic) - 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.
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® 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.

Date 18 Mar 2024

Nancy Considine
Quality Approver