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New England Biolabs
Certificate of Analysis

Product Name:	*Thermolabile USER® II Enzyme*
Catalog Number:	*M5508S*
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 fluorescently labeled oligonucleotide duplex containing a single uracil base in 15 minutes at 37°C in a total reaction volume of 10 µL in 1X T4 DNA Ligase Buffer.*
Lot Number:	*10011779*
Expiration Date:	*06/2020*
Storage Temperature:	*-20°C*
Storage Conditions:	*25 mM KCl, 35 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 15 mM Tris-HCl, 100 µg/ml BSA, 50 % Glycerol, (pH 7.5 @ 25°C)*
Specification Version:	*PS-M5508S/L v1.0*

Thermolabile USER® II Enzyme Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M5508SVIAL	Thermolabile USER® II Enzyme	10011780	**Pass**
B7204SVIAL	CutSmart® Buffer	3081804	**Pass**

Assay Name/Specification	**Lot # 10011779**
qPCR DNA Contamination (E. coli Genomic) A minimum of 1 unit of Thermolabile USER® II 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**
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 Thermolabile USER® II Enzyme is incubated at 37ºC. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	**Pass**
Functional Testing (Thermolability, Endonuclease III) A 10 µl reaction in CutSmart® Buffer containing 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base and 1 unit of Thermolabile USER® II Enzyme was incubated for 15 minutes at 37ºC followed by heat	**Pass**

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Assay Name/Specification	Lot # 10011779
inactivation for 10 minutes at 65ºC. The addition of 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single AP site and incubation for 15 minutes at 37ºC followed by 10 minutes at 75ºC, results in no cleavage of additional substrate.	
Functional Testing (Thermolability, UDG) A 10 µl reaction in CutSmart® Buffer containing 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base and 1 unit of Thermolabile USER® II Enzyme was incubated for 15 minutes at 37ºC followed by heat inactivation for 10 minutes at 65ºC. The addition of 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base with 20 units of Endonuclease III and incubation for 15 minutes at 37ºC followed by 10 minutes at 75ºC, results in no cleavage of additional substrate.	**Pass**
Functional Testing (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 Thermolabile USER® II 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**

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

Lauren Sears Higgins
Production Scientist
19 Jun 2018

Josh Hersey
Packaging Quality Control Inspector
06 Jul 2018