

## New England Biolabs Certificate of Analysis

*Product Name:* Thermolabile USER<sup>®</sup> II Enzyme  
*Catalog #:* M5508S/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 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 #:* 0011709  
*Assay Date:* 09/2017  
*Expiration Date:* 9/2019  
*Storage Temp:* -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  
*Effective Date:* 08 Aug 2017

Assay Name/Specification (minimum release criteria)	Lot #0011709
<p><b>Functional Testing (Thermolability, Endonuclease III)</b> - A 10 µl reaction in CutSmart<sup>®</sup> Buffer containing 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base and 1 unit of Thermolabile USER<sup>®</sup> II Enzyme was incubated for 15 minutes at 37°C followed by heat inactivation for 10 minutes at 65°C. The addition of 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single AP site and incubation for 15 minutes at 37°C followed by 10 minutes at 75°C, results in no cleavage of additional substrate.</p>	<b>Pass</b>
<p><b>Functional Testing (Thermolability, UDG)</b> - A 10 µl reaction in CutSmart<sup>®</sup> Buffer containing 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base and 1 unit of Thermolabile USER<sup>®</sup> II Enzyme was incubated for 15 minutes at 37°C followed by heat inactivation for 10 minutes at 65°C. 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 37°C followed by 10 minutes at 75°C, results in no cleavage of additional substrate.</p>	<b>Pass</b>
<p><b>Functional Testing (USER, Transformation assay)</b> - A 10 µl reaction in ThermoPol<sup>®</sup> Reaction Buffer containing 20 ng linearized pNEB206A, 100 ng of a 950 bp control PCR product and 1 unit of Thermolabile USER<sup>®</sup> II 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>



