

## New England Biolabs Certificate of Analysis

**Product Name:** *Thermolabile USER® II Enzyme*  
**Catalog Number:** *M5508L*  
**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:** *10054424*  
**Expiration Date:** *08/2021*  
**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
M5508LVIAL	Thermolabile USER® II Enzyme	10054425	Pass
B7204SVIAL	CutSmart® Buffer	10046082	Pass

Assay Name/Specification	Lot # 10054424
<p><b>qPCR DNA Contamination (E. coli Genomic)</b>            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.</p>	Pass
<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 Thermolabile USER® II 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>	Pass
<p><b>Functional Testing (Thermolability, Endonuclease III)</b>            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 37°C followed by heat</p>	Pass

