

## New England Biolabs Product Specification

**Product Name:** *Thermostable USER<sup>®</sup> III Enzyme*

**Catalog #:** *M5509S*

**Concentration:** *1,000 units/ml*

**Unit Definition:** *One unit is defined as the amount of enzyme required to nick 10 pmol of a 60-mer fluorescently labeled oligonucleotide duplex containing a single uracil base in 15 minutes at 65°C in a total reaction volume of 10 µL in 1X ThermoPol<sup>®</sup> Buffer.*

**Shelf Life:** *24 months*

**Storage Temp:** *-20°C*

**Storage Conditions:** *10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 178 µg/ml BSA (pH 7.4 @ 25°C)*

**Specification Version:** *PS-M5509S v1.0*

**Effective Date:** *05 Dec 2022*

### Assay Name/Specification (minimum release criteria)

**qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 1 unit of Thermostable USER<sup>®</sup> III Enzyme is screened for the presence of *E. coli* genomic DNA using SYBR<sup>®</sup> 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  $\leq 1$  *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 Thermostable USER<sup>®</sup> III 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.

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Doreen Duquette  
Quality Approver

