240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

New England Biolabs Product Specification

Product Name: Thermolabile USER® 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.

Shelf Life: 24 months
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)

Functional Testing (Thermolability, Endonuclease III) - A 10 μ l 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 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.

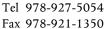
Functional Testing (Thermolability, UDG) - A 10 μ l reaction in CutSmart® Buffer containing 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base and 1 unit of Thermolabile USER® II Enzyme was incubated for 15 minutes at 37°C followed by heat inactivation for 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.

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.









www.neb.com info@neb.com

New England Biolabs Product Specification

Assay Name/Specification (minimum release criteria)

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.

RNase Activity (Extended Digestion) - A 10 μ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 μ l of Thermolabile USER® II 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.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Kuk Cotum

Date 08 Aug 2017

Derek Robinson Quality Approver





