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## New England Biolabs Certificate of Analysis

Product Name: Topoisomerase I (E. coli)

Catalog #: M0301S/L
Concentration: 5,000 units/ml

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the relaxation of > 95% of 0.5  $\mu$ g of negatively supercoiled pUC19

RF I DNA in a total reaction volume of 25  $\mu$ l in 15 minutes at 37°C.

 Lot #:
 0081803

 Assay Date:
 03/2018

 Expiration Date:
 03/2019

 Storage Temp:
 -20°C

Storage Conditions: 10 mM Tris-HCl, 50 mM KCl, 35 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.5 @ 25°C)

Specification Version: PS-M0301S/L v1.0
Effective Date: 03 Aug 2016

Assay Name/Specification (minimum release criteria)	Lot #0081803
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 μl reaction in CutSmart® Buffer containing 1 μg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 50 units of Topoisomerase I ( <i>E. coli</i> ) incubated for 4 hours at 37°C releases <0.5% of the total radioactivity.	Pass
<b>qPCR DNA Contamination</b> ( <i>E. coli</i> <b>Genomic</b> ) - A minimum of 5 units of Topoisomerase I ( <i>E. coli</i> ) is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is $\leq 1$ <i>E. coli</i> genome.	Pass
RNase Activity (Extended Digestion) - A 10 $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ l of Topoisomerase I ( <i>E. coli</i> ) 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.	Pass

Authorized by Derek Robinson 03 Aug 2016







Inspected by
Cathy Shea
02 Mar 2018