

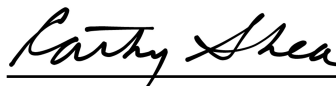
## New England Biolabs Certificate of Analysis

*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 µg of negatively supercoiled pUC19 RF I DNA in a total reaction volume of 25 µl in 15 minutes at 37°C.  
*Lot #:* 0081708  
*Assay Date:* 08/2017  
*Expiration Date:* 8/2018  
*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 #0081708
<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.	<b>Pass</b>
<b>qPCR DNA Contamination (<i>E. coli</i> 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 ≤ 1 <i>E. coli</i> genome.	<b>Pass</b>
<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 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.	<b>Pass</b>



Authorized by  
Derek Robinson  
03 Aug 2016



Inspected by  
Cathy Shea  
29 Aug 2017

