

## New England Biolabs Certificate of Analysis

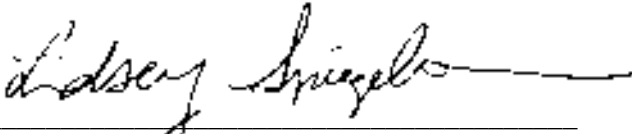
**Product Name:** *Topoisomerase I (E. coli)*  
**Catalog Number:** M0301S  
**Concentration:** 5,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme that catalyzes the relaxation of > 95% of 0.5 µg of negatively supercoiled pUC19 RF I DNA in a total reaction volume of 25 µl in 15 minutes at 37°C.  
**Packaging Lot Number:** 10192458  
**Expiration Date:** 05/2024  
**Storage Temperature:** -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

Topoisomerase I (E. coli) Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0301SVIAL	Topoisomerase I (E. coli)	10187334	Pass
B6004SVIAL	rCutSmart™ Buffer	10182170	Pass

Assay Name/Specification	Lot # 10192458
<p><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] E. coli DNA and a minimum of 50 units of Topoisomerase I (E. coli) incubated for 4 hours at 37°C releases &lt;0.5% of the total radioactivity.</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 Topoisomerase I (E. coli) 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>qPCR DNA Contamination (E. coli Genomic)</b>            A minimum of 5 units of Topoisomerase I (E. coli) 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

This product has been tested and shown to be in compliance with all specifications.

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