

New England Biolabs Product Specification

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| Product Name: | <i>Topoisomerase I (E. coli)</i> |
| 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. |
| Shelf Life: | 12 months |
| Storage Temp: | -20°C |
| Storage Conditions: | 10 mM Tris-HCl, 50 mM KCl, 35 mM (NH ₄) ₂ SO ₄ , 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)

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in CutSmart® Buffer containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 50 units of Topoisomerase I (*E. coli*) incubated for 4 hours at 37°C releases <0.5% of the total radioactivity.

qPCR DNA Contamination (*E. coli* Genomic) - 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.

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 Topoisomerase I (*E. coli*) 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.



Date 03 Aug 2016

Derek Robinson
Director of Quality Control

