

## Topoisomerase I (*E. coli*)



1-800-632-7799  
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# M0301S



**100 units**      **5,000 U/ml**      **Lot: 0081509**

**RECOMBINANT**    **Store at -20°C**    **Exp: 9/16**

**Description:** Topoisomerase I (*E. coli*) catalyzes the relaxation of negatively supercoiled DNA. Topoisomerase I has also been implicated in knotting and unknotting DNA (1) and in linking complementary rings of single-stranded DNA into double-stranded rings (2). The intact holoenzyme is a 97 kDa protein.

**Source:** An *E. coli* strain containing the cloned topA gene from *E. coli*.

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 35 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

**Reagents Supplied with Enzyme:**  
10X CutSmart® Buffer.

**Reaction Conditions:** 1X CutSmart Buffer.  
Incubate at 37°C.

**1X CutSmart Buffer:**  
50 mM potassium acetate  
20 mM Tris acetate  
10 mM magnesium acetate  
100 µg/ml BSA  
pH 7.9 @ 25°C

**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. DNA supercoiling is assessed by agarose gel electrophoresis in the absence of ethidium bromide.

**Quality Assurance:** Purified free of contaminating exonucleases and endonucleases.

### Quality Control Assays:

**16-Hour Incubation:** A 50 µl reaction containing 1 µg of λ DNA/HindIII fragments and 50 units of Topoisomerase I incubated for 16 hours resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Exonuclease Activity:** Incubation of 50 units of Topoisomerase I with 1 µg sonicated single and doubled-stranded [<sup>3</sup>H] DNA (200,000 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.5% radioactivity.

**Endonuclease Activity:** Incubation of 40 units of Topoisomerase I with 1 µg pUC19 DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II, as determined by agarose gel electrophoresis in the presence of ethidium bromide.

### Enzyme Properties

#### Activity in NEBuffers:

NEBuffer 1	25%
NEBuffer 2	50%
NEBuffer 3	0%
NEBuffer 4	100%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

**Survival in a Reaction:** A minimum of 0.5 unit is required to digest 1 µg of substrate DNA in 16 hours.

**Heat Inactivation:** 40 units of Topoisomerase I were inactivated by incubation at 65°C for 20 minutes.

#### References:

1. Liu, L.F. et al. (1976) *J. Mol. Biol.* 106, 439–452.
2. Kirkegaard, K. and Wang, J.C. (1978) *Nucleic Acids Res.* 5, 3811–3820.

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