Topoisomerase I
(E. coli)

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M0301S 008121013101

M0301S

RR NEB4 BSA 37° Yes

100 units **5,000 U/ml** **Lot: 0081210**
Recombinant **Store at –20°C** **Exp: 10/13**

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 $(NH_4)_2SO_4$, 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris acetate
10 mM magnesium acetate
1 mM DTT
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 [^{3}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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CERTIFICATE OF ANALYSIS