Topoisomerase I (E. coli)

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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 (NH₄)₂SO₄, 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 double-stranded [*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 agarase 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:

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