

***E. coli* Topoisomerase IV Microplate Assay Kit**



Product Description (Product Numbers TRIV01 and TRIV02)

The kit is supplied with sufficient topo IV enzyme, plasmid DNA substrate (supercoiled pNO1; supplied at 1 mg/ml), 5X Assay Buffer, Enzyme Dilution Buffer, DNA-detection dye (Promega Diamond dye) and TFO1 oligo for 100 assays. The enzyme is supplied at a concentration of 10 U/ μ l in Dilution Buffer (see Note 1). The kit is also supplied with sufficient Wash Buffer, TF buffer and T10 buffer for one 96-well plate. These buffers are supplied as 20 X concentrates and must be diluted prior to use with ultra-pure water. Store enzyme at -80°C. (Stable for 6 months undiluted). The enzyme should be centrifuged briefly before opening and it is recommended that it is aliquoted (undiluted) to avoid repeated freeze-thaw cycles.

For *in vitro* laboratory research use only.

Dilution Buffer

40 mM HEPES.KOH (pH 7.6)
100 mM potassium glutamate
1 mM DTT
1 mM EDTA
40 % (v/v) glycerol

Assay Buffer (supplied as 5X stock)

40 mM HEPES.KOH (pH 7.6)
100 mM potassium glutamate
10 mM magnesium acetate
10 mM DTT
1 mM ATP
50 μ g/ml albumin

TF Buffer (supplied as a 20X stock)

50 mM sodium acetate (pH 5.0)
50 mM NaCl
50 mM MgCl₂

Wash Buffer (supplied as a 20X stock)

20 mM Tris.HCl (pH 7.6)
137 mM NaCl
0.005 % (w/v) BSA
0.05 % (v/v) Tween-20

DNA-detection Dye (supplied as a 1,000X stock)

Supplied in 100% DMSO

T10 Buffer (supplied as a 20X stock)

10 mM Tris.HCl (pH 8)
1 mM EDTA

Preparation of Plate and Relaxation Assay

Rehydrate wells with 3 x 200 μ l Wash Buffer (diluted from 20X stock before use).

Immobilize 100 μ l of 500nM TFO1 oligo in each well (5 μ l of 10 μ M TFO1 in 95 μ l Wash Buffer), 5 minutes at room temperature. Wash off excess oligo with 3 x 200 μ l Wash Buffer.

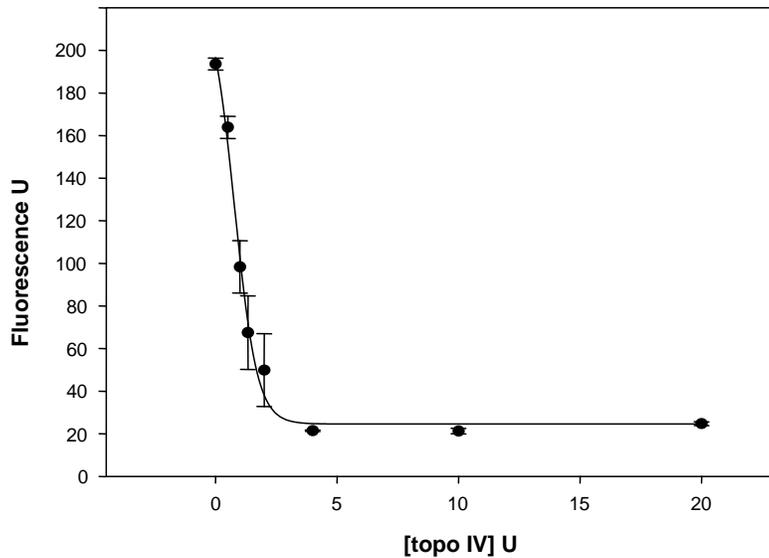
Incubate 1.5 U of topo IV with 0.75 μ g of supercoiled pNO1 in a reaction volume of 30 μ l at 37°C for 30 minutes in Assay Buffer. Incubate reaction in well.

Add 100 μ l TF Buffer (diluted from 20X stock before use) to well and incubate for a further 30 minutes at room temperature to allow triplex formation.

Remove liquid from well and wash with 3 x 200 μ l TF Buffer to remove unbound plasmid.

Stain with DNA-detection dye (diluted to 1X with T10 buffer. Add 200 μ l per well. Incubate for 10 - 20 minutes, **mix** and read in fluorescence plate reader; Ex: 495 nm; Em: 537 nm).

Relaxation of pNO1 by varying amounts of topo IV



Quality Control

Purity: The A and B subunits are purified to >95% purity as judged by SDS-polyacrylamide gel electrophoresis.

Endonuclease assay: 0.5 μ g supercoiled pBR322 incubated with 1 U topo IV for 1 hour at 37°C in the presence of 1 mM ATP shows no detectable conversion of superhelical DNA to either open circular or linear forms when assayed by agarose gel electrophoresis.

References

Maxwell, A., Burton, N.P. and O'Hagan, N. (2006) High-throughput assays for DNA gyrase and other topoisomerases. *Nucleic Acid Res.* **34(15)**, e104

Notes.

1. A unit of enzyme activity is defined as the amount of enzyme that just supercoils 0.5 μ g of pNO1 in 30 minutes at 37°C as judged by agarose gel electrophoresis.
2. It is important to mix the samples before reading or they will be greatly reduced.

**Patent for assay held by Inspiralis Ltd., Norwich, Norfolk, UK. (Patent No. GB0424953.8).
Kit issued with limited licence for individual use only.**

DNA-detection stain is Diamond nucleic acid dye supplied under license from Promega Corporation.