

Human Topoisomerase II Microplate Assay Kit



Product Description (Product Number TRTA201 and TRTB201)

The kit is supplied with sufficient human topo II enzyme, plasmid DNA substrate (supercoiled pNO1; supplied at 1 mg/ml), 10X Assay Buffer, ATP, Enzyme Dilution Buffer, DNA-detection dye (Promega Diamond dye) and TFO1 oligo for 100 assays. The enzyme is supplied at a minimum 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 20X concentrates and must be diluted prior to use with ultra-pure water.

Store at -80 °C. (Stable for 3 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

50 mM Tris.HCl (pH 7.5)
100 mM NaCl
1 mM DTT
0.5 mM EDTA
50 % (v/v) glycerol
50 μ g/ml albumin

Assay Buffer (supplied as 10X stock)

50 mM Tris.HCl (pH7.5)
125 mM NaCl
10 mM MgCl₂
5 mM DTT
100 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

ATP (supplied as 30X stock)

30 mM ATP

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).

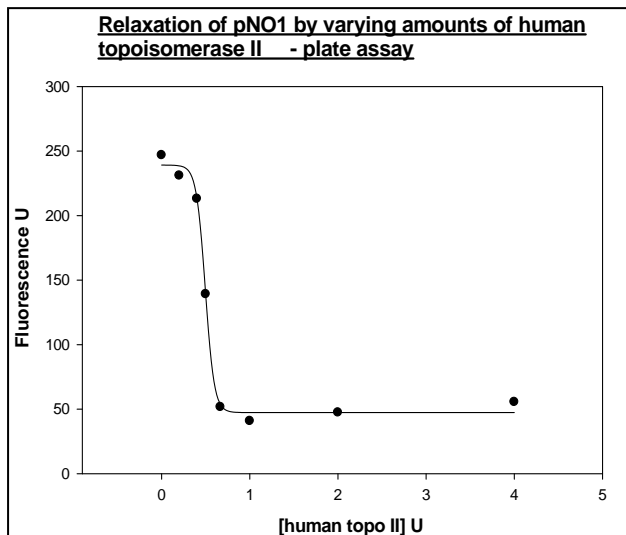
Immobilize 100 μ l of 500nM TFO1 oligo in well (5 μ l of 10 μ M TFO1 in 95 μ l Wash Buffer), 5 minutes at room temperature. Wash off excess oligo with 3 x 200 μ l Wash Buffer.

Incubate 1.5 U of human topo II 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).



Quality Control

1) Purity: Human topoisomerase II is purified to > 95 % purity as judged by SDS-polyacrylamide gel electrophoresis. 2) Tests for human topoisomerase I contamination by looking for relaxation of supercoiled pBR322 under topoisomerase I assay conditions were negative. 3) kDNA or pBR322 were also incubated for 4 hrs in assay buffer + 10 mM MgCl₂ at 37 °C. These tests were negative for the formation of linear products, indicating the absence of nuclease contamination.

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 relaxes 0.5 µg 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.