

# Human Topoisomerase II Microplate Assay Kit



## Product Description (Product Number TRT201 and TRT202)

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 and TFO1 oligo for 100 assays. The enzyme is supplied at a concentration of 10 U/μl in Dilution Buffer. 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.) It is recommended that the enzyme is aliquoted 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 μg/ml albumin

### Assay Buffer (supplied as 10X stock)

50 mM Tris.HCl (pH7.5)  
125 mM NaCl  
10 mM MgCl<sub>2</sub>  
5 mM DTT  
100 μg/ml albumin

### TF Buffer (supplied as a 20X stock)

50 mM sodium acetate (pH 5.0)  
50 mM NaCl  
50 mM MgCl<sub>2</sub>

### Wash Buffer (supplied as a 20X stock)

20 mM Tris.HCl (pH 7.6)  
137 mM NaCl  
0.01 % (w/v) BSA  
0.05 % (v/v) Tween-20

### ATP (supplied as 30X stock)

30 mM ATP

### 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 μl Wash Buffer (diluted from 20X stock before use).

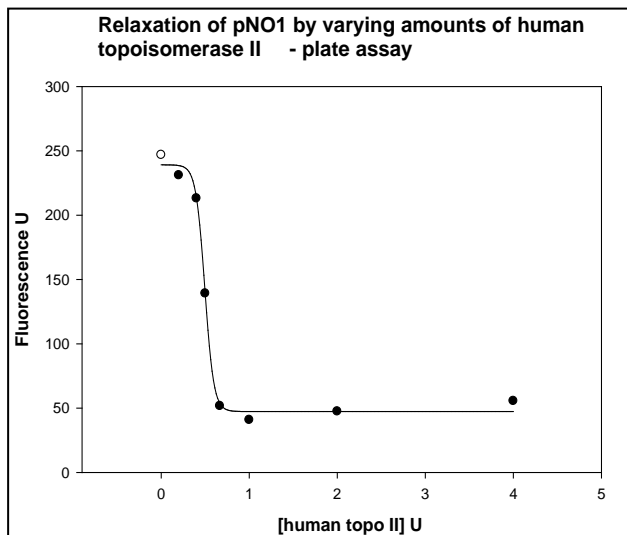
Immobilize 100 μl of 500nM TFO1 oligo in well (5 μl of 10 μM TFO1 in 95 μl Wash Buffer), 5 minutes at room temperature. Wash off excess oligo with 3 x 200 μl Wash Buffer.

Incubate 1.5 U of human topo II with 0.75 μg of supercoiled pNO1 in a reaction volume of 30 μl at 37°C for 30 minutes in Assay Buffer. Incubate reaction in well.

Add 100 μl 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 μl TF Buffer to remove unbound plasmid.

Stain with appropriate fluorescence stain (Suggested stain, SYBR Gold<sup>®</sup> (Invitrogen) diluted to 1X with T10 buffer. Add 200 μl 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 MgCl<sub>2</sub> 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

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