

# ***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 and TFO1 oligo for 100 assays. The enzyme is supplied at a concentration of 10 U/ $\mu$ l 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 20 X concentrates and must be diluted prior to use with ultra-pure water.

Store enzyme at  $-80^{\circ}\text{C}$ . (Stable for 6 months undiluted)

**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  $\mu$ g/ml albumin

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

50 mM sodium acetate (pH 5.0)  
50 mM NaCl  
50 mM  $\text{MgCl}_2$

### **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

### **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  $\mu$ l Wash Buffer (diluted from 20X stock before use).

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

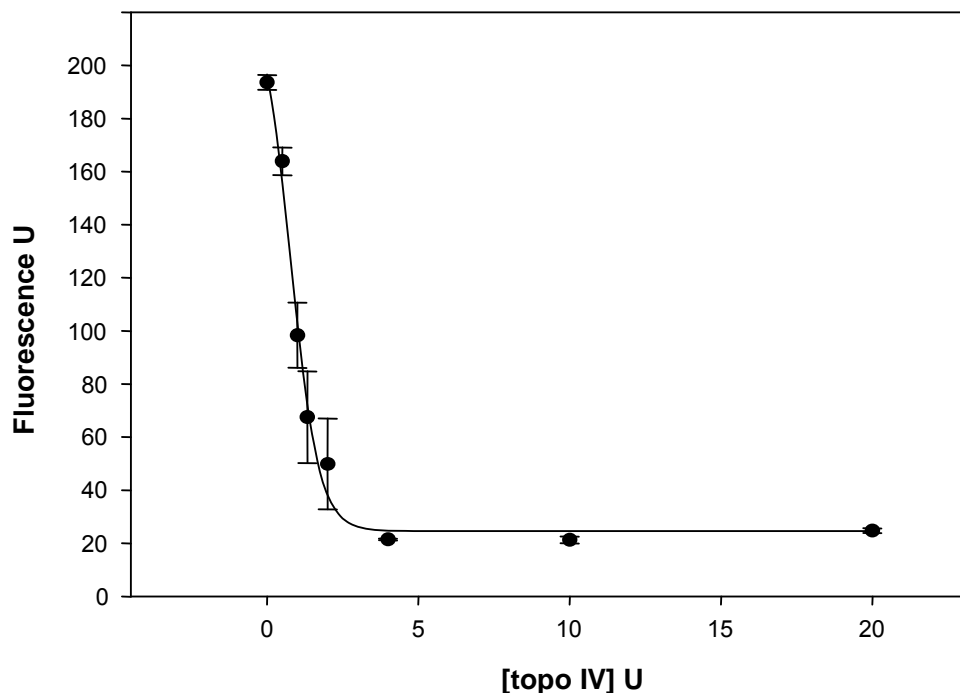
Incubate 1.5 U of topo IV with 0.75  $\mu$ g of supercoiled pNO1 in a reaction volume of 30  $\mu$ l at  $37^{\circ}\text{C}$  for 30 minutes in Assay Buffer. Incubate reaction in well.

Add 100  $\mu$ 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  $\mu$ l 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  $\mu$ 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  $\mu$ 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

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