

***E. coli* Gyrase Microplate Assay Kit**



Product Description (Product Number TRG01 and TRG02)

The kit is supplied with sufficient gyrase enzyme, plasmid DNA substrate (relaxed 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 5 U/ μ 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 at -80°C . (Stable for 6 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 KCl
2 mM DTT
1 mM EDTA
50 % (w/v) glycerol

Assay Buffer (supplied as 5X stock)

35 mM Tris.HCl (pH 7.5)
24 mM KCl
4 mM MgCl_2
2 mM DTT
1.8 mM spermidine
1 mM ATP
6.5 % (w/v) glycerol
0.1 mg/ml albumin

TF Buffer

(supplied as a 20X stock)

50 mM sodium acetate (pH 5.0)
50 mM NaCl
50 mM 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 Supercoiling 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.

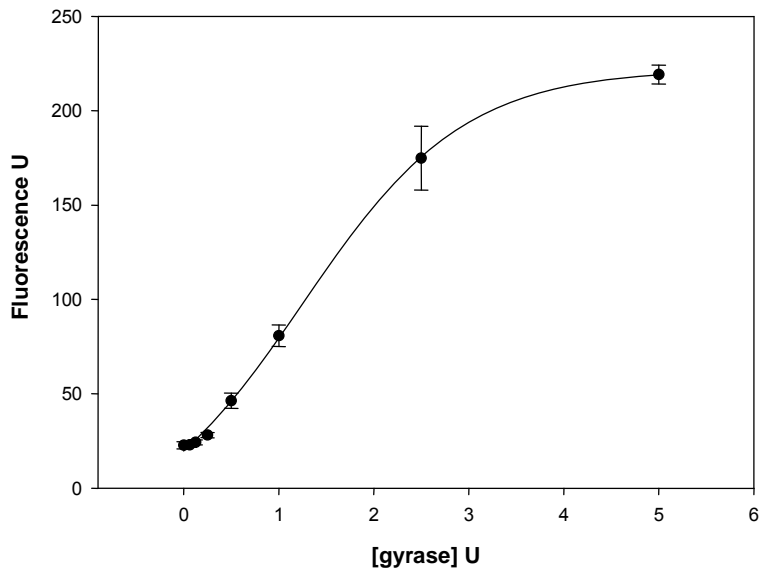
Incubate 1.5 U of DNA gyrase with 0.75 μ g of relaxed pNO1 in a reaction volume of 30 μ l at 37°C for 30 minutes in Assay Buffer. Incubate reaction in well of plate.

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 appropriate fluorescence stain (Suggested stain, SYBR Gold[®] (Invitrogen) 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.)

Supercoiling of pNO1 by varying amounts of gyrase



Quality Control

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

Endonuclease assay: 0.5 µg supercoiled pBR322 incubated with 1 U of DNA gyrase 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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