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, DNA-detection dye (Promega Diamond dye) and TFO1 oligo for 100 assays. The enzyme is supplied at a concentration of 5 U/µl in Dilution Buffer (see Note 1). However, we recommend that the enzyme is titrated into the assay to ascertain the minimum volume of enzyme required per assay to achieve full relaxation. Particularly if the kit is being used for drug screening purposes. 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). The enzyme should be centrifuged briefly before opening and it is recommended that it is aliquoted (undiluted) multiple freeze-thaw cycles are likely to happen.

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
0.005 % (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 Supercoiling Assay

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

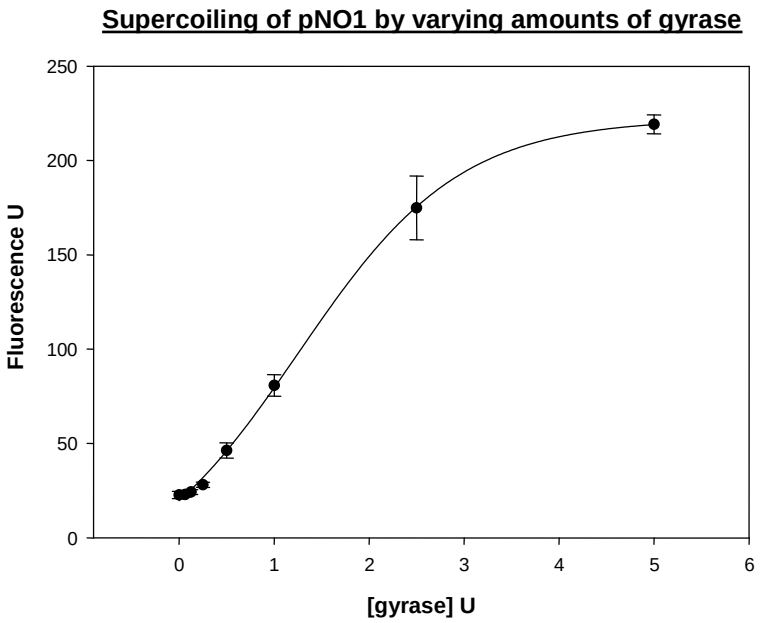
Immobilise 100 µl of 500nM TFO1 oligo in each well (5 µl of 10 µM TFO1 in 95 µl Wash Buffer), 5 minutes at room temperature. Wash off excess oligo with 3 x 200 µl Wash Buffer.

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

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 DNA-detection dye (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

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 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

Notes.

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