S. aureus Gyrase Microplate Assay Kit

Product Description (Product Number # SATRG01, SATRG02)

The kit is supplied with sufficient *S. aureus* 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 10 U/µl 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)
1 mM DTT
1 mM EDTA
40 % (w/v) glycerol

Assay Buffer
(supplied as 5X stock)

40 mM HEPES.KOH (pH 7.6)
10 mM magnesium acetate
10 mM DTT
2 mM ATP
500 mM potassium glutamate
0.05 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).

Immobilize 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.

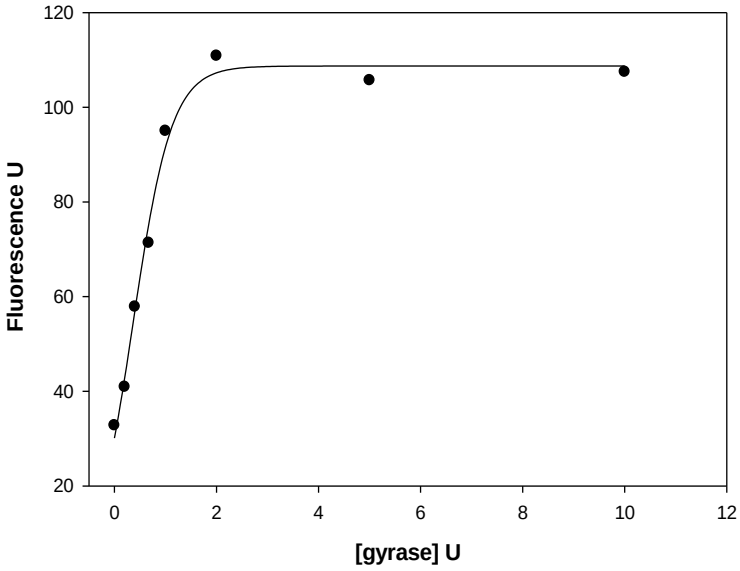
Incubate 1.5 U of *S. aureus* gyrase with 0.75 µg of relaxed pNO1 in a reaction volume of 30 µl at 37°C for 30 minutes in Assay Buffer. Incubate reaction in well.

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)..

Supercoiling of relaxed pNO1 by varying amounts of *S. aureus* gyrase in the plate assay



Quality Control

1) Purity: *S. aureus* gyrase is purified to > 95 % purity as judged by SDS-polyacrylamide gel electrophoresis.
2) pBR322 was also incubated for 4 hrs in assay buffer + 10 mM $MgCl_2$ at 37 °C. These tests were negative for the formation of linear products, indicating the absence of nuclease contamination.
(3) No activity was detectable when the single subunits were assayed alone.

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 relaxed pBR322 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.