Pseudomonas aeruginosa DNA Gyrase Supercoiling Assay Kits

Product Description (Product Numbers PAS001, PAS002 and PAS003)

P. aeruginosa gyrase is prepared by overexpressing the GyrA and GyrB subunits in *E. coli* and purified using in-house methods. It is supplied in Dilution Buffer as a heterotetramer complex at a minimum concentration of 10s U/µl (please see Certificate of Analysis for specific lot numbers). However, we recommend that the enzyme is titrated into the assay to ascertain the minimum volume of enzyme required per assay to achieve full supercoiling particularly if the kit is being used for drug screening purposes. Please refer to the protocol P.aeruginosa-Gyrase-Supercoiling-Assay-Protocol.pdf (inspiralis.com). Kits are supplied with enzyme, assay and dilution buffers and relaxed pBR322 substrate. Store at -80 °C. For larger kits it is recommended that the enzyme is aliquoted to avoid repeated freeze-thaw cycles.

For *in vitro* laboratory research use only.

Dilution Buffer (1X)

50 mM Tris.HCl (pH 7.6)
50 mM sodium chloride
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 potassium chloride
4 mM magnesium chloride
2 mM DTT
1.8 mM spermidine
1 mM ATP
6.5 % (w/v) glycerol
0.1 mg/ml albumin

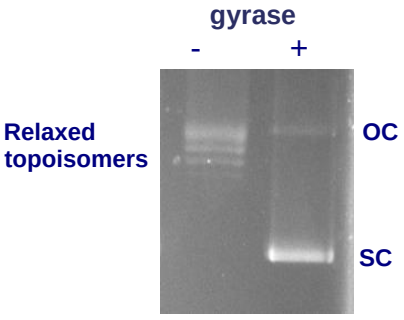
pBR322 Storage Buffer

10 mM Tris.HCl (pH 7.5)
1 mM EDTA

Supercoiling Assay

1 U of gyrase will fully supercoil 0.5 µg of relaxed pBR322 is incubated in 1X assay buffer in a total reaction volume of 30 µl at 37°C for 30 minutes. Gels are run in the absence of intercalators (e.g. ethidium bromide or chloroquine).

OC=nicked, open circular, SC=supercoiled



Quality Control

1) Purity: *P. aeruginosa* gyrase is purified to > 95 % purity as judged by SDS-polyacrylamide gel electrophoresis.
2) No activity was detectable when the single subunits were assayed alone