E. coli Gyrase Supercoiling Assay Kit

Product Description (Product Numbers K0001, K0002, K0003 and K0004)

DNA gyrase (isolated from *E.coli*) is prepared from the over-expressing strains JMtacA and JMtacB (Hallett *et al*., 1990) and is supplied as an A_2B_2 complex.

The enzyme is supplied at a minimum concentration of 10 U/µl in Dilution Buffer. 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 for more information:
https://www.inspiralis.com/assets/TechnicalDocuments/E.coli-Gyrase-Supercoiling-Assay-Protocol.pdf

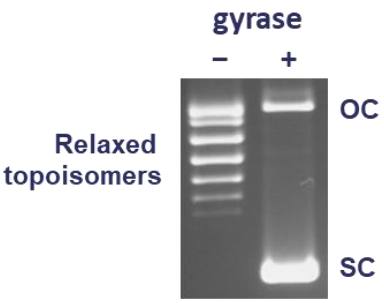
Store at -80 °C. It is recommended that the enzyme is aliquoted to avoid repeated freeze-thaw cycles.
For *in vitro* laboratory research use only.

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



Supercoiling Assay

1 U of DNA gyrase is incubated with 0.5 µg of relaxed pBR322 in a reaction volume of 30 µl at 37 °C for 30 minutes in Assay Buffer.
Gels are run in the absence of ethidium bromide or chloroquine.

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 relaxed pBR322 incubated with 0.5 U of DNA gyrase for 1 hour at 37 °C in the presence of 1 mM ATP shows no detectable conversion of supercoiled DNA to either open circular or linear forms when assayed by agarose gel electrophoresis.

Reference

Hallett, P., Grimshaw, A.J., Wigley, D.B. and Maxwell, A. (1990). Cloning of the DNA gyrase genes under *tac* promoter control: overproduction of the gyrase A and B proteins. *Gene* 93: 139-142