E. coli Gyrase Assay Kit for Cell Extracts

Product Description (Product Numbers GCE001, GCE002, GCE003 and GCE004)

Contains 5X Assay Buffer, Dilution Buffer, relaxed pBR322 DNA and supercoiled pBR322 marker. Store at -20°C.

For *in vitro* laboratory research use only.

Dilution Buffer

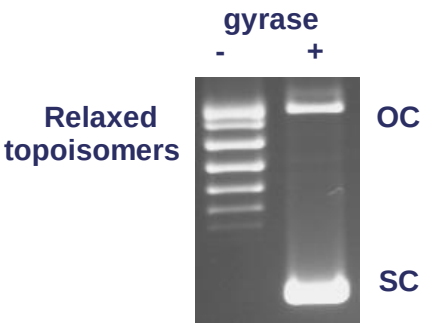
50 mM Tris.HCl (pH 7.5)
100 mM KCl
2 mM DTT
1 mM EDTA
50 % (w/v) glycerol

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. (For cell extracts use increased dilutions to avoid contaminating nuclease activity)
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