E. coli DNA Gyrase Assay and Dilution Buffers

Product Description (Product Numbers B0001 and B0002)

For use with *E. coli* DNA gyrase enzyme and over expressed cell extracts containing DNA gyrase. Store at -20 °C.

For *in vitro* laboratory research use only.

Dilution Buffer

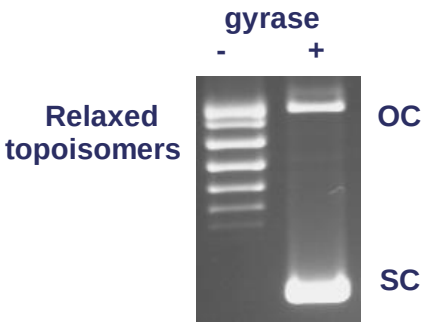
50 mM Tris.HCl (pH 7.5)
100 mM KCl
2 mM dithiothreitol
1 mM EDTA
50 % (w/v) glycerol

Assay Buffer (supplied as 5x stock)

35 mM Tris.HCl (pH 7.5)
24 mM KCl
4 mM $MgCl_2$
2 mM dithiothreitol
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 superhelical 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