E. coli DNA Gyrase Cleavage Assay Kit

Product Description

(Product Numbers GCK001, GCK002, GCK003 and GCK004)

E. coli DNA gyrase is prepared from the overproducing strains JMtacA and JMtacB (Hallett *et al*., 1990) and is supplied as an A_2B_2 complex. The enzyme is supplied at a concentration of 3.5 µM in Dilution Buffer and is suitable for cleavage assays. Cleavage activity is a minimum of 2.5 – 5.0 U/µl.
50 % cleavage can be obtained with 0.2 µl in the presence of 10 µM CFX in a 30 µl reaction (see typical titration below).

Store at -80°C. **For *in vitro* laboratory research use only.**

Dilution Buffer

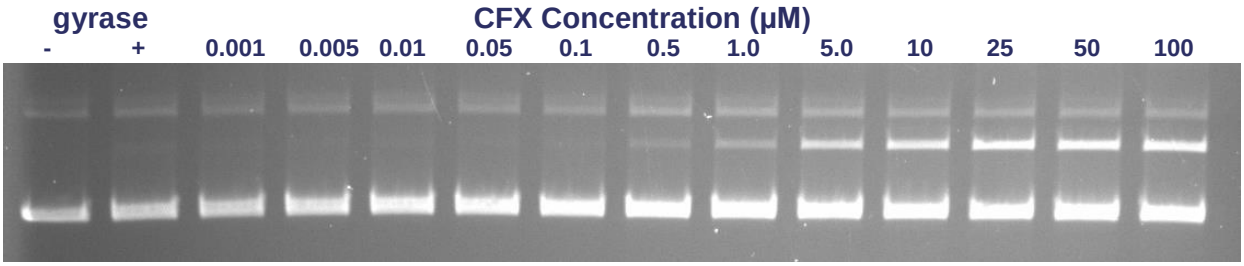
50 mM Tris.HCl (pH 7.5)
100 mM KCl
2 mM DTT
1 mM EDTA
50 % (w/v) glycerol

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
6.5 % (w/v) glycerol
0.1 mg/ml albumin

Cleavage Assay

DNA gyrase is incubated with 0.5 µg of supercoiled pBR322 in a reaction volume of 30 µl at 37 °C for 1 hour in Assay Buffer in the presence of CFX. 0.2 % SDS and 0.1 mg/ml Proteinase K are added before a further incubation at 37 °C for 30 minutes.



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