

# *E. coli* Gyrase Cleavage Assay Kit



## Product Description (Product Numbers GCK001, GCK002, GCK003 and GCK004)

*E. coli* gyrase is prepared from the overproducing strains JMtacA and JMtacB (Hallett *et al.*, 1990) and is supplied as an A<sub>2</sub>B<sub>2</sub> complex. The enzyme is supplied at a concentration of 2.4 μM in Dilution Buffer and is suitable for cleavage assays. Supercoiling activity is 50 U/μl. Cleavage activity is 4 U/μl.

80 % cleavage can be obtained with 0.25 μl (24 nM) in the presence of 5 μM CFX in a 30 μl reaction (see 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)

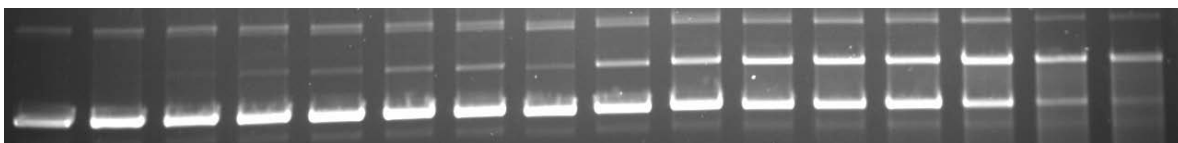
35 mM Tris.HCl (pH 7.5)  
24 mM KCl  
4 mM MgCl<sub>2</sub>  
2 mM DTT  
1.8 mM spermidine  
6.5 % (w/v) glycerol  
0.1 mg/ml albumin

### Cleavage Assay

Gyrase is incubated with 0.3 μg of supercoiled pBR322 in a reaction volume of 30 μl at 37 °C for 1 hour in Assay Buffer. 0.2 % SDS and 0.1 mg/ml Proteinase K are added before a further incubation at 37 °C for 30 minutes.

### Titration of DNA Gyrase (nM) under Cleavage Assay Conditions (5μM CFX)

- 0.4 0.8 1.2 1.6 2.0 2.4 4.0 8.0 12 16 20 24 40 80 120 nM



### 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