

# ***E. coli* Gyrase Supercoiling Assay Kit**



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

Gyrase (isolated from *E. coli*) is prepared from the overexpressing 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 5 U/μl in Dilution Buffer.

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

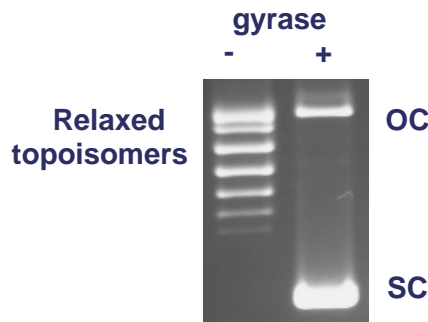
### **Assay Buffer (supplied as 5X stock)**

35 mM Tris.HCl (pH 7.5)  
24 mM KCl  
4 mM MgCl<sub>2</sub>  
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 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