

# Escherichia coli Gyrase



## Product Description (Product Numbers # G1001, G5002, G1003, G2004)

DNA gyrase is prepared from the over producing 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

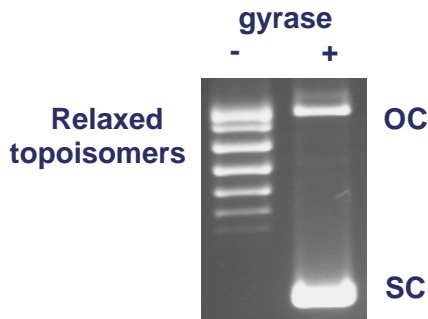
50 mM Tris.HCl (pH 7.5)  
100 mM KCl  
2 mM DTT  
1 mM EDTA  
50 % (w/v) glycerol

### 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 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