

# *E. coli* Gyrase Assay Kit for Cell Extracts



## Product Description (Product Numbers GCE001, GCE002, GCE003 and GCE004)

Contains 5X Assay Buffer, Dilution Buffer, relaxed pBR322 DNA and supercoiled pBR322 marker. Store at -20°C.

**For *in vitro* laboratory research use only.**

### Dilution Buffer

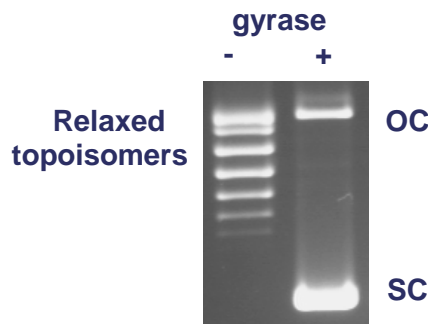
50 mM Tris.HCl (pH 7.5)  
100 mM KCl  
2 mM DTT  
1 mM EDTA  
50 % (w/v) glycerol

### 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. (For cell extracts use increased dilutions to avoid contaminating nuclease activity)  
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