

# C.difficile Gyrase Assay And Dilution Buffers



## Product Description (Product Numbers CDB001 and CDB002)

For use with *C. difficile* gyrase enzymes and over expressed cell extracts containing gyrase. Store at -20 °C. It is recommended that the buffer 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)

50 mM Tris.HCl (7.5)  
5 mM MgCl<sub>2</sub>  
5 mM DTT  
1.5 mM ATP  
350 mM potassium glutamate  
0.05 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 supercoiled DNA to either open circular or linear forms when assayed by agarose gel electrophoresis.