

## C.difficile Gyrase



### Product Description (Product Numbers CDG001 and CDG002)

*C. difficile* DNA gyrase (isolated from *E. coli*) is protein purified after heterologous expression in *E. coli* and is supplied as an A<sub>2</sub>B<sub>2</sub> complex. The enzyme is supplied at a minimum 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)

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.