

Quinolone Resistant *E. coli* Gyrase



Product Description (Product Number AS83W001)

Quinolone resistant gyrase is prepared from the overproducing strains JMtacA and JMtacB (Hallett, *et al.*, 1990) where JMtacA contains the Ser83Trp point mutation and is supplied as an A₂B₂ complex. The enzyme is supplied at a concentration of 10 U/μl in Dilution Buffer. This mutant confers 50 -100X more resistance to quinolones than the wild type, with a CFX IC₅₀ of 50 μM compared to 500 nM for wt.

Store at -80°C.

For *in vitro* laboratory research use only.

Dilution Buffer

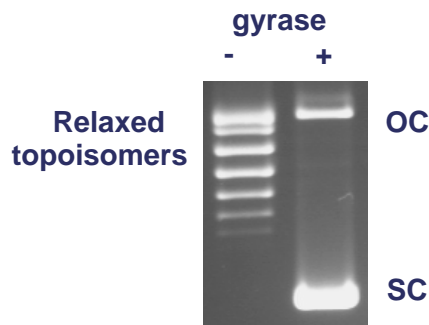
50 mM Tris.HCl (pH 7.5)
100 mM KCl
2 mM DTT
1 mM EDTA
50 % (w/v) glycerol

Assay Buffer (supplied as 5x stock)

35 mM Tris.HCl (pH 7.5)
24 mM KCl
4 mM MgCl₂
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 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