TopoGEN
Dedicated to Studies of Topoisomerase Enzymes

Purified E. coli DNA Gyrase **[TG2000G-1, TG2000G-3, TG2000G-5, TG2000G-7]**

Product Description
Contains purified bacterial (E. coli) DNA Gyrase purified to homogeneity (on SDS-PAGE). DNA Gyrase is prepared from overexpressing strains and is supplied as purified holoenzyme in an A2B2 complex. The enzyme is supplied at the unit concentration given on the above sticker (in storage buffer: 50 mM Tris-Cl pH 7.5, 100 mM KCl, 2 mM dithiotreitol, 1 mM EDTA, 50% glycerol).
Other purified topoisomerases and antibodies are available from TopoGEN and may be ordered on line at www.topogen.com.

Storage and Shipping Conditions
The active gyrase should be stored at –70 C and is stable undiluted for at least 6 months in this concentrated state. The enzyme can be aliquoted on first thawing to minimize damage from multiple freeze thaw cycles.

Unit Defintion
One unit of gyrase will supercoil 0.5 ug of plasmid in 60 min under conditions described below.

DNA Gyrase Quality Control Tests
1. A test for nuclease contamination was carried out by assaying for the formation of linear KDNA and linear plasmid DNA. Incubations of 1 µg of catenated KDNA or supercoiled pUC19 DNA (4 hrs. at 37° in the presence of 10 mM MgCl2) were performed. Linear DNA or breakdown products were not generated under these conditions.
2. The A and B subunits are >95% pure based upon SDS-PAGE and certified to be endonuclease free.

Dilution Buffer
Dilutions should be performed in 50 mM Tris-Cl (pH 7.5), 100 mM KCl, 2 mM dithiothreitol, 1 mM EDTA, 50% Glycerol.

Supercoiling Assay Conditions
One unit of gyrase is incubated with 0.5 ug of relaxed plasmid DNA in a reaction volume of 30 ul for 1 hr. at 37 C in assay buffer. Agarose gels are run in the absence of ethidium bromide. One unit of gyrase will supercoil 0.5 ug of plasmid in 1 hr. under these conditions.
Assay buffer (1x) constituents:
35 mM Tris-Cl pH 7.5
24 mM KCl
4 mM MgCl2
2 mM dithiothreitol
1.8 mM spermidine
1 mM ATP
6.5% glycerol
0.1mg BSA/ml
(note that the assay buffer is supplied as a 5x stock and the above formula is for 1x)

References
Hallett, P. et al. (1990) Cloning of DNA Gyrase Genes Under Tac Promoter Control: Overproduction of Gyrase A and B Proteins. Gene ***93***:139-142.

TopoGEN, Inc. 108 Aces Alley, Port Orange, Florida 32128, USA. **Tel:** 614-451-5810, **Fax:** 614-559-3932
Email: Sales: info@topogen.com, **Support:** support@topogen.com, **Website:** www.topogen.com