



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

This enzyme 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 Definition

One unit of gyrase will supercoil 0.2 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.

Product Application and Disclaimer

This product is not licensed or approved for administration to humans or animals. It may be used with experimental animals only. The product is for in vitro research diagnostic studies only. The product is non-infectious and non-hazardous to human health. This information is based on present knowledge and does not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship. TopoGEN, Inc. shall not be held liable for product failure due to mishandling and incorrect storage by end user. TopoGEN's liability is limited to credit or product replacement.