TopoGEN

Research, Diagnostic and Therapeutic
Biomedical Reagents, Kits and Services
www.topogen.com

Purified S. aureus DNA Gyrase Enzyme

- TG2000GSA-1 [100 units]
  TG2000GSA-3 [500 units]
  TG2000GSA-5 [1000 units]
  TG2000GSA-7 [2000 units]

Product Description

DNA gyrase is a type II topoisomerase encoded by two genes *GyrA* and *GyrB* from *S. aureus*. DNA gyrase is an essential topoisomerase that supercoils DNA through a process of strand breakage/resealing and DNA wrapping (3). As a type II enzyme, gyrase is unique in its ability to negatively supercoil a relaxed plasmid DNA substrate. DNA gyrase is also the target for quinolone-based antibacterial agents which act by subverting the enzyme into a DNA damaging agent. TopoGEN offers purified DNA gyrase from the gram positive bacterium, *Staphylococcus aureus* for use in all aspects of drug development and screening assays. The enzyme is very active in decatenating and supercoiling KDNA (TG2013) but will also negatively supercoil relaxed plasmid DNA (TG2035). The *S. aureus* enzyme is a heterotetramer of GyrA2GyrB2 and is purified as His tagged subunits that are reassembled to make active enzyme (4). Other purified topoisomerases and antibodies are available from TopoGEN and may be ordered on line at www.topogen.com.

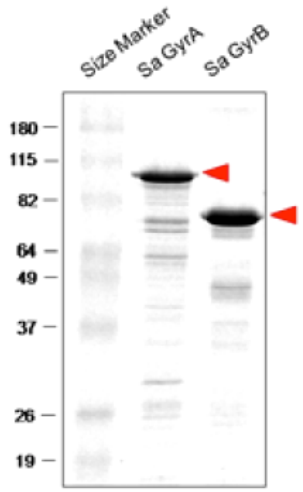
Storage and Shipping Conditions

This enzyme should be stored at –20°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 will supercoil 100 ng of plasmid in 30 min at 37°C under conditions described below. DNA gyrase will also decatenate kDNA, like all type II enzymes (3); however, the product will be negatively supercoiled minicircular kDNA.

DNA Gyrase Quality Control Tests



SDS PAGE of Gyrase

1. DNA gyrase subunits were cloned, overexpressed and purified using the proprietary company methods. A single band on SDS-PAGE was detected by CB staining for each subunit. Cross contamination by topo I was assessed by assaying for relaxation of supercoiled DNA under conditions optimized for type I activity. Under these conditions, after 2 hours of incubation with supercoiled plasmid DNA, no relaxation products were detectable.
2. 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 DNA (4 hrs. at 37°C in the presence of 10 mM MgCl2) were performed. Linear DNA or breakdown products were not generated under these conditions.

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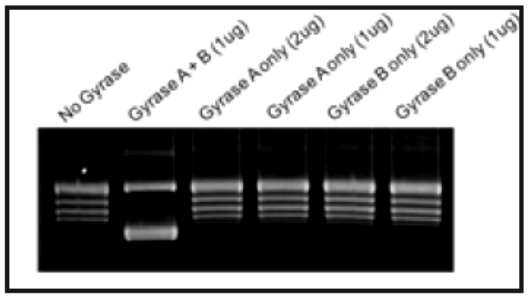


3. The subunits are better than 95% pure based upon SDS-PAGE. Neither subunit is active in the absence of the other and neither subunit displays nucleolytic activity. These data show that host (E. coli) is not contributing to the activity of individual subunits of S. aureus gyrA or B. This was confirmed by Western blotting probings with anti-GyrA IgG specific to E. coli (data not shown).

Assay Conditions

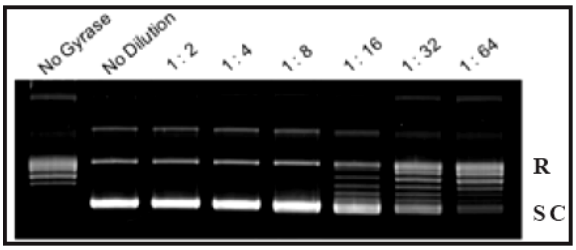
TopoGEN provides samples of *three buffers that are used to assay S. aureus DNA Gyrase. The buffers are:

5X Assay Stock (0.5 ml provided) contains the following solutes:

375 mM Tris-HCl (pH 7.5)
37.5mM MgCl2
37.5mM DTT
0.375 mg/ml BSA
150mM KCl

10X ATP Stock: 20 mM ATP (0.25 mL). Enzyme requires 2.0 mM ATP FINAL CONCENTRATION.

5X Potassium Glutamate Stock: 2.5M (0.25 mL). Enzyme requires 500 mM K-Glu FINAL CONCENTRATION.
**Note: These buffers are provided to allow the customer to establish a working control for the Gyrase assay. Additional buffer may be required and the customer is advised to follow the above recipes for replenishment. Store all buffers at -20°C.*



Assays should be performed in a final volume of 20 ul by adding appropriate amounts of buffers, DNA enzyme (add enzyme last to initiate the assay). Typical assay would be:

Distilled water:	8ul
5X Assay stock:	4ul
10X ATP Stock:	2ul
5X K-Glu Stock:	4ul
Relaxed Plasmid DNA	1ul
Purified enzyme (variable)	1ul Added last

(Enzyme diluted to working concentration using dilution buffer or titrate as 2 fold serial dilutions.)
Incubate 30 min 37°C, stop by addition of SDS to 1% (not included); add Bromophenol blue/glycerol loading dye, run 1% agarose gel with relaxed/supercoiled markers. Optional: digest with proteinase K and repurify DNA by phenol extraction. Gels should be stained with Ethidium Bromide (0.5 ug/ml 20-30 min), destained in water for 15-30 min at room temperature and photodocumented.

Dilution buffer

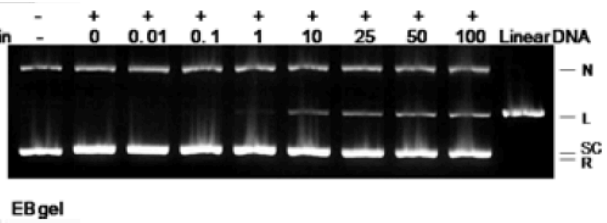
If necessary, dilute the enzyme using the following buffer (1x) supplied with the enzyme.
50 mM Tris-HCl [pH 7.5], 100 mM NaCl, 2mM b- DTT, 1 mM EDTA, 10% glycerol.

Modifications of the Gyrase Assay: Detecting DNA cleavages.

Gyrase is a target for several antibiotics that induce the enzyme to cleave the plasmid DNA substrate (3,5). Quinolone drugs are known to induce cleavages concurrent with covalent complex formation; thus, to detect such complexes in drug screening experiments, it is essential that proteinase K digestions be carried out and that reaction products repurified by phenol/chloroform extraction. Additionally, the conditions that are optimal for cleavage detection, differ slightly from those optimized for catalytic assays. For DNA Gyrase, we find that reduced potassium glutamate (200 mM final concentration) is ideal for cleavage complex formation.

The 1x cleavage buffer contains:

75mM Tris-HCl (pH 7.5)
7.5mM MgCl2
7.5mM DTT
2 mM ATP
75 ug/ml BSA
30mM KCl
200mM K-Glu



Typical Gyrase Cleavage Assay Result with Ciprofloxacin. Reactions were carried out using 4 units of S. aureus Gyrase and the indicated concentrations of ciprofloxacin (ug/mL). Gel was 1% agarose containing 0.5 ug EB/ml in buffer and gel. Note that the supercoiled plasmid DNA and relaxed DNA are poorly resolved in this gel system. N, nicked DNA; L, linear DNA; SC, supercoiled DNA; R, relaxed DNA.

To resolve nicked and linear DNA cleavages, we recommend running 1% agarose gels containing 0.5 u/ml EB in the gel and running buffer. Destain for 15 min prior to photodocumenting the results. It is essential to include linear DNA markers in these gels.

Contents shipped with Enzyme

1. 5 X Gyrase Assay Buffer (0.5mL)
2. 10X ATP Buffer (0.25 mL)
3. 2.5 M K-Glutamate (0.25 mL)
4, Dilution Buffer for Gyrase (0.5mL)

References

1. Kato et al. (1990) Cell 63:393-404.
2. Peng, H., and Marians, K. (1999). DNA Topoisomerase Protocols Volume I; page 163 (available from www.topogen.com)
3. Wang, J. (1996) DNA Topoisomerases. Ann. Rev. Biochem 65:635-692
4. Levine et al., (1998) Biochem Biophys Acta 1400:29-43
5. Strahilevitz, J and Hooper, D. (2005) 49:1946-1956