



Purified E. coli DNA Gyrase and Relaxed DNA Kit

	<i>Enzyme (Units):</i>	<i>DNA (ug):</i>
<input type="checkbox"/> <i>Catalog No. 2000G-1KIT</i>	<i>100</i>	<i>50</i>
<input type="checkbox"/> <i>Catalog No. 2000G-3KIT</i>	<i>500</i>	<i>250</i>
<input type="checkbox"/> <i>Catalog No. 2000G-5KIT</i>	<i>1000</i>	<i>500</i>
<input type="checkbox"/> <i>Catalog No. 2000G-7KIT</i>	<i>2000</i>	<i>1000</i>



Gyrase QC



DNA QC

Kit Description

This kit 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 QC sticker (in storage buffer: 50 mM Tris-HCl pH 7.5, 100 mM KCl, 2 mM dithiothreitol, 1 mM EDTA, 50% glycerol). Also included is substrate DNA (relaxed pBR-322) supplied in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5).

Other purified topoisomerases and antibodies are available from TopoGEN and may be ordered online at www.topogen.com

Storage and Shipping Conditions

The active gyrase should be stored at -20°C and is stable for at least 6 months in this concentrated state. The enzyme should be aliquoted on first thawing to minimize damage from multiple freeze thaw cycles. Store the relaxed DNA at 4°C (stable for 6 months).

Unit Definition

One unit of gyrase will supercoil 0.5 ug of plasmid in 1 hr. at 37°C under conditions described below.

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 in 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.

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For Technical Questions:

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DNA Gyrase/Relaxed DNA Catalog # 2000G-KIT

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 NaCl, 2 mM dithiothreitol, 1 mM EDTA and 50% glycerol.

Supercoiling Assay Conditions

One unit of gyrase is incubated with 0.5 µg of relaxed plasmid DNA in a reaction volume of 30 µl for 1 hr. at 37°C in assay buffer^a. Agarose gels are run in the absence of ethidium bromide. One unit of gyrase will supercoil 0.5 µg of plasmid in 1 hr. under these conditions.

^aAssay buffer (1x recipe is shown below; kit includes a 5x stock based on this formula):

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

0.1 mg BSA/ml

Relaxed DNA Quality Control Tests:

1. Purity was evaluated spectrophotometrically using A₂₆₀:A₂₈₀ readings.
2. Incubations with gyrase buffer at 37°C for 60 min did not result in formation of nicked or linear DNA species.
3. Relaxation of supercoiled DNA was carried out using TopoGEN's purified DNA topoisomerase I (Catalog # 2005H, available online at www.topogen.com). Reactions were terminated by heating to 75°C and the topological state confirmed by agarose gel electrophoresis.

Kit Contents and assay protocol: Volumes are given for the 2000G-1KIT size. (For the larger kits size, multiply volumes by 5, 10 or 20 fold as appropriate, see page 1 for Kit sizes).

- Relaxed pBR-322 DNA (50 ug total) DNA concentration is specified on page 1 (usually 250 ng/ul).*
- Supercoiled pBR-322 DNA (25 ul in gel loading buffer). Load 2 ul as a marker.*
- 5x Gyrase assay buffer (600 ul) 1x buffer contains recipe is given above.*
- Dilution buffer (600 ul). Dilute gyrase with this buffer. Recipe for dilution buffer given above.*
- 5x Stop Buffer/gel loading dye (600 ul): 5x buffer is 5% Sarkosyl, 0.125% bromophenol blue, 25% glycerol.*
- Purified DNA gyrase (unit definition given on page 1).*

Protocol Summary: Reaction volumes should be 20-30 ul final volume (generally limited by volume that can be loaded onto the gel). Reactions are assembled in microfuge tubes with water, buffer and substrate pBR-322 relaxed DNA. The gyrase should be added last and the reactions incubated at 37°C for 15-60 min. (or longer as appropriate) then terminated with the stop/load buffer, proteinase K digested, extracted and loaded onto an agarose gel (note, DO NOT add ethidium bromide (EB) to gel or gel buffer during electrophoresis, use EB to stain the gel AFTER electrophoretic separation).

Sample reaction (20 ul, order of addition is shown):

Distilled water-----14 ul (Vary as needed to bring volume up to 20 ul final)

5x Assay Buffer----- 4 ul

pBR-322 Relaxed DNA----- 1 ul (vary as needed to give 100-400 ng as appropriate)*

*The amount of relaxed pBR322 DNA depends on sensitivity and detection methods. With routine staining using ethidium bromide, about 250 ng of DNA is usually sufficient; however, less may be used with more sensitive stains.

Gyrase ----- 1 ul (diluted as appropriate, see unit definition page 1)

- Incubate 60 min at 37°C.*
- Add 1/5 vol of stop buffer/loading dye*
- Add proteinase K to 50 ug/ml, digest for 10-30 min at 37°C (optional step).*
- Add 20 ul of Chloroform:isoamyl alcohol (24:1 mixture), vortex briefly, withdraw blue, aqueous phase.*
- Load blue phase onto a 1% agarose (50x TAE buffer: 242 g Tris base, 57.1 ml glacial acetic acid, 100 ml 0.5M EDTA). DO NOT INCLUDE ETHIDIUM BROMIDE IN THE GEL OR BUFFER AT THIS STEP.*
- Electrophorese until dye travels to the bottom of the gel.*
- Stain with 0.5 ug/ml ethidium bromide for 30 min (caution, EB is a mutagen, WEAR GLOVES).*
- Destain (distilled water) for 10-30 min room temperature (handle gel with gloves).*
- Photograph using UV transilluminator.*

Analysis of reaction products by electrophoresis: For each gel, run a negative control (relaxed DNA lacking gyrase). The negative control should look very different from the gyrase reaction products (which will be faster migrating, supercoiled DNA forms). *Important: Failure to detect gyrase activity may indicate that the 5x buffer is inactive; thus, remake this buffer and repeat the assay to ensure that the ATP is still useable. The 5x buffer can degrade over time.*

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

Trask and Muller (1983) Biochemical characterization of topo I from avian erythrocytes. *Nuc. Acid. Res.* 11:2779

Boros, Posfai, and Venetianer (1984) High copy derivatives of pBR-322 *Gene* 30:257

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