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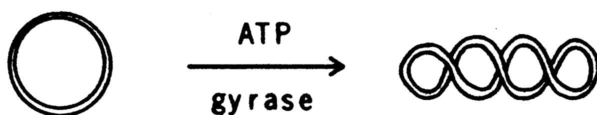
Product Information

DNA Gyrase
from *E. coli*

Catalog Number **D0690**
Storage Temperature –70 °C

EC 5.99.1.3

Product Description
DNA gyrase belongs to the type II topoisomerase family, which catalyzes DNA topological transformation by transiently cleaving both strands of a DNA duplex in concert to form an enzyme-opened gate. Only gyrase can introduce negative supercoils, but both gyrase and topoisomerase II can decatenate DNA. These enzymes are closely related, and mutagenesis experiments have successfully converted DNA gyrase into a type II topoisomerase.[1] DNA gyrase catalyzes the ATP-dependent introduction of negative supercoils into relaxed DNA.[2]



This product is supplied in a solution of 50 mM Trizma®-HCl, pH 7.5, 0.1 M KCl, 1 mM EDTA, 2 mM dithiothreitol and 50% glycerol. It is isolated from an overexpressing strain of *E. coli*.[3]

DNA gyrase is supplied as an A_2B_2 holoenzyme. The reported molecular mass of the complex is ~374 kDa, with the A subunit of 97 kDa, and the B subunit of 90 kDa.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The enzyme is shipped on dry ice and should be stored at –70 °C. The enzyme should be aliquoted after the first thaw, as repeated rounds of freeze/thaw will lead to loss of activity. Do **not** store in a frost-free freezer. When on ice, the enzyme activity is stable for 1-3 days (not longer). In general, the enzyme is less stable when diluted.

Procedure
A suggested reaction buffer for this product is as follows:
- 35 mM Trizma-HCl, pH 7.5
- 25 mM KCl
- 4 mM $MgCl_2$
- 2 mM dithiothreitol (DTT)
- 1.8 mM spermidine
- 1 mM ATP
- 6.25% (w/v) glycerol
- mg/mL bovine serum albumin (BSA)

DNA gyrase can be assayed by following either its ability to decatenate DNA, or its ability to introduce negative supercoils in an ATP-dependent fashion.[4] High salt concentrations inhibit the enzyme. Therefore, samples should not contribute greater than 30 mM monovalent salt to the final reaction. If the ion concentration exceeds this, then a low salt buffer should be used to compensate. A negative control should always be run to determine if the results are due to sample-induced, altered electrophoretic mobility of DNA. If this is the case, it may be necessary to phenol/chloroform-treat and/or ethanol-precipitate the completed assay reactions before loading the gels.

Serious complications can arise when interfering proteins or substances are present in the samples. Inhibitors of gyrase include natural products.[5] In addition, crude cell-free extracts may contain large concentrations of DNA-binding proteins or positively charged proteins that stick to the DNA and inhibit enzyme access. Additionally, nuclease contaminants may degrade or nick DNA substrates and obscure the results. By diluting extracts and/or adding a tRNA carrier to compete with basic proteins, one can sometimes minimize such problems. Should this fail, the sample may require further purification, such as ammonium sulfate precipitation followed by desalting column chromatography.

Product Profile

Unit Definition: One unit of gyrase will supercoil 0.2 μg of plasmid DNA in 30 minutes at 37 °C.

Purity: >95% (SDS-PAGE, and free of detectable contaminating proteins)

Nuclease contamination is assayed with a test for linear KDNA and linear plasmid DNA formation. 1 μg of either catenated KDNA or supercoiled pUC19 DNA is incubated with DNA gyrase for 4 hours at 37 °C, in the presence of 10 mM $MgCl_2$.

References

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2. Wang, J., *Annu. Rev. Biochem.*, **65**, 635-692 (1996).
3. Hallett, P. *et al.*, *Gene*, **93(1)**, 139-142 (1990).
4. Marini, J.C. *et al.*, *J. Biol. Chem.*, **255(11)**, 4976-4979 (1980).
5. Maxwell, A. *Biochem. Soc. Trans.*, **27(2)**, 38-53 (1999).

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