

DNA Gyrase (*E. coli*)



1-800-632-7799
info@neb.com
www.neb.com



M0306S 002140115011

M0306S



100 units Lot: 0021401 Exp: 1/15
5,000 U/ml Store at -70°C

Description: DNA Gyrase (*E. coli*) is a Type II topoisomerase that catalyzes the introduction of negative supercoils in DNA in the presence of ATP. The gyrase holoenzyme is a heterotetramer made up of 2 gyrA (97 kDa) subunits and 2 gyrB (90 kDa) subunits.

Source: An *E. coli* strain containing the cloned and expressed gyrA and gyrB genes.

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Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 2 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
5X DNA Gyrase (*E. coli*) Reaction Buffer

Reaction Conditions: 1X DNA Gyrase (*E. coli*) Reaction Buffer. Incubate at 37°C .

1X DNA Gyrase (*E. coli*) Reaction Buffer:

35 mM Tris-HCl
24 mM KCl
4 mM MgCl₂
2 mM DTT
1.75 mM ATP
5 mM spermidine
0.1 mg/ml BSA
6.5% glycerol
pH 7.5 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the conversion of DNA Gyrase (*E. coli*) Substrate (NEB #N0471) to > 95% of 0.5 μg of supercoiled plasmid in a total reaction volume of 30 μl in 30 minutes at 37°C . DNA supercoiling is assessed by agarose gel electrophoresis in the absence of ethidium bromide.

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Quality Control Assays:

16-Hour Incubation: A 50 μl reaction containing 1 μg of λ DNA (HindIII digest) and 25 units of DNA Gyrase (*E. coli*) for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 μl reaction containing 25 units of DNA Gyrase (*E. coli*) with 1 μg of a mixture of single and double-stranded [³H] *E. coli* DNA (200,000 cpm/ μg) for 4 hours at 37°C in released < 0.5% of the total radioactivity.

Endonuclease Activity: Incubation of 25 units of DNA Gyrase (*E. coli*) with 1 μg pUC19 DNA for 4 hours at 37°C in 50 μl reaction buffer resulted in < 10% conversion to RF II.

Enzyme Properties

Survival in a Reaction: A minimum of 0.13 unit is required to convert 0.5 μg of substrate DNA to supercoil plasmid in a total reaction volume of 30 μl in 16 hours.

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Heat Inactivation: 25 units of DNA Gyrase (*E. coli*) were inactivated by incubation at 65°C for 20 minutes.

Note: The 5X DNA Gyrase Reaction Buffer should be stored at -70°C to maintain optimal stability of components.

References:

1. Adachi, T. et al. (1987) *Nucleic Acids Res.* 15, 771–784.
2. Higgins, N.P. et al. (1978) *PNAS* 75, 1773–1777.
3. Swanberg, S.L. and Wang, J.C. (1987) *J. Mol. Biol.* 197, 729–736.

Companion Product:

DNA Gyrase (*E. coli*) Substrate
#N0471S 20 μg

CERTIFICATE OF ANALYSIS

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