DNA Gyrase (*E. coli*)

NEW ENGLAND BioLabs Inc.
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M0306S 002140815081

M0306S

RR 37° Yes

100 units **Lot: 0021408** **Exp: 8/15**

5,000 U/ml **Store at –80°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.

Note: Store at –80°C

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 $MgCl_2$
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.

Quality Control Assays:

16-Hour Incubation: A 50 µl reaction containing 1 µg of λDNA (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 [^{3}H] *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 sustrate DNA to supercoil plasmid in a total reaction volume of 30 µl in 16 hours.

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 –80°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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