DNA Gyrase

(E. coli)

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 survival in a reaction containing 25 units of DNA Gyrase (E. coli) with 1 µg of a mixture of single and double-stranded [³²P] E. coli DNA (200,000 cpm/µg) for 4 hours at 37°C in released < 0.5% of the total radioactivity.

Exonuclease Activity:
Incubation of a 50 µl reaction containing 25 units of DNA Gyrase (E. coli) with 1 µg pUC19 DNA for 4 hours at 37°C in released < 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.

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:

Companion Product:
DNA Gyrase (E. coli) Substrate #N0471S 20 µg