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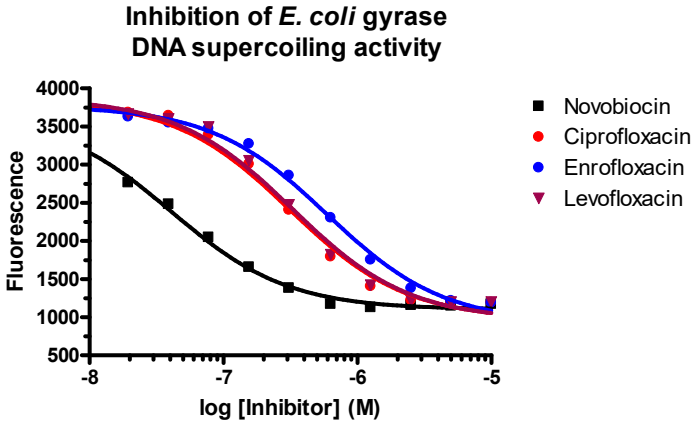
INSTRUCTIONS

ProFoldin
E. coli DNA Gyrase

E. coli DNA Gyrase – for 100 assays Catalog No. TOP2-100EC

Protein construct:	Wild-type *E. coli* DNA gyrase, the $(gyrA)_2(gyrB)_2$ complex, composed of the gyrA subunit (MW 97 kDa) and gyrB subunit (90 kDa) purified from a bacterial expression system.
MW:	374 kDa
Enzyme concentration:	2 µM
Enzyme activity assay:	The DNA gyrase DNA supercoiling activity is measured by using the DNA Topoisomerase II (Gyrase) Assay Kit (Catalog No. DSA020K)
Storage temperature:	-20 or -80°C. Do not freeze-and-thaw repeatedly.
Enzyme dilution:	Use the 1 x assay to dilute the enzyme just before the assay. Do not store diluted enzyme solution



The ***E. coli* DNA Topoisomerase II (Gyrase) – for 100 assays** (Catalog No. TOP2-100EC) 50 µl 100 x *E.coli* gyrase (2 µM). It is for 100 assays.

Assay Protocol using DNA Topoisomerase II (Gyrase) Assay Kit

The following assay protocol is based on a 96-well assay plate format using a standard black 96-well plate (Greiner 655076). For 384-well plate assays using a standard black 384-well plate (Matrix 4318), please reduce the reagent volumes proportionally.

1. Reaction:

The total volume of each reaction mixture is 40 µl including: 24 µl of H_2O, 4 µl of 10 x buffer, 4 µl of 10 x relaxed DNA (250 µg/ml relaxed plasmid DNA), 4 µl of 10 x enzyme, 4 µl of 10 mM ATP. Incubate the reaction mixture at 37°C for 60 min.

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Note: The final concentrations are 20 mM Tris-HCl, pH 8, 35 mM NH_4OAc, 4.6 % glycerol, 1 mM DTT, 0.005% Brij35, 8 mM $MgCl_2$, 25 μg/ml relaxed plasmid DNA, 1 mM ATP and 20 nM topoisomerase II. The 10 x enzyme is prepared by dilution of the 100x enzyme in 1x assay buffer. A negative control reaction can be the reaction mixture without addition of ATP. Magnesium is essential for the reaction and assay. EDTA should be avoided.

2. Assay

(1) Make 1 x H19 Dilution Buffer by dilution of the 10 x H19 Dilution Buffer 10-fold with water. Freshly prepare the H19 dye by dilution of 1 μl the 1500 x H19 dye stock solution with 1.5 ml of 1 x H19 Dilution Buffer (1500 x dilution). Mix the solution evenly by inverting the tube a few times.

(2) Mix 250 μl of the freshly prepared H19 dye with each reaction solution (40 μl). Incubate the mixture at room temperature for 5 min.

(3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Related Products:

Human Topoisomerase I, 10,000 Units	HTOPI-010
DNA Topoisomerase II (Gyrase) Assay Kit	DSA020K
E. coli DNA Topoisomerase II (Gyrase) Assay Kit Plus-100	DSA100KE
Gel-based *E. coli* DNA Topoisomerase II (Gyrase) Assay Kit Plus-100	GDSA100KE
Relaxed plasmid DNA - 0.050 mg	RDNA-050UG
Supercoiled plasmid DNA - 0.050 mg	SDNA-050UG
H19 Dye for DNA Relaxation and Supercoiling Assays	DSA1000D
Human Topoisomerase I DNA Relaxation Assay Kit Plus -100	HRA100KE
Human Topoisomerase II DNA Decatenation Assay Kit Plus-100	HDC100KE
E. coli DNA Topoisomerase I Assay Kit Plus-100	DRA100KE
E. coli gyrase ATPase assay Kit Plus	T2A-100KE
96-well Topoisomerase DNA Cleavage Assay Kit	TDC96K
96-Well Topoisomerase DNA Decatenation Assay Kit	TDD96K
96-Well *S. aureus* Topo IV DNA Decatenation Assay Kit Plus	SDD96KE

Publications

1. Asha M.K. et al, In vitro anti-Helicobacter pylori activity of a flavonoid rich extract of Glycyrrhiza glabra and its probable mechanisms of action, Journal of Ethnopharmacology, Vol.145(2), pp.581-586 (2013).
2. Mora-Pale M et al, Antimicrobial mechanism of resveratrol-trans-dihydrodimer produced from peroxidase-catalyzed oxidation of resveratrol. Biotechnol Bioeng.Vol 112, pp2417–2428 (2015).

For more information of DNA topoisomerase assays and assays for more drug targets and enzymes, please visit www.profoldin.com or send emails to info@profoldin.com.