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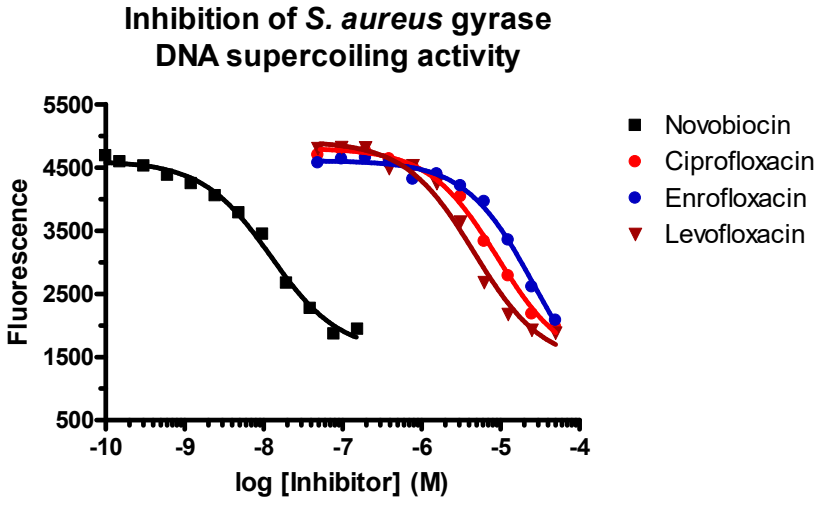
INSTRUCTIONS

ProFoldin
S. aureus DNA Gyrase

S. aureus DNA Gyrase – for 100 assays Catalog No. TOP2-100SA

Protein construct:	Wild-type *S. aureus* DNA gyrase, the $(gyrA)_2(gyrB)_2$ complex, composed of the gyrA subunit (MW 99 kDa) and gyrB subunit (73 kDa) purified from a bacterial expression system.
MW:	344 kDa
Enzyme concentration:	7.5 µM
Enzyme activity assay:	The DNA gyrase DNA supercoiling activity is measured by using the *S. aureus* DNA Topoisomerase II (Gyrase) Assay Kit (Catalog No. DSA100KS).
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 ***S. aureus* DNA Gyrase – for 100 assays** (Catalog No. TOP2-100SA) includes 43 µl 7.5 uM *S. aureus* gyrase. It is for 100 assays in a 96-well plate format.

Assay Protocol using the *S. aureus* 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.

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1. Reaction:

The total volume of each reaction mixture is 40 µl including: 19.6 µl of H_2O, 4 µl of 10 x buffer, 8 µl of 2 M potassium glutamate, 4 µl of 10 x relaxed DNA (250 µg/ml), 0.4 µl of 7.5 µM *S. aureus* gyrase, 4 µl of 10 mM ATP. Incubate the reaction mixture at 37°C for 60 min.

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 $MgCl_2$, 400 mM potassium glutamate, 25 µg/ml relaxed plasmid DNA, 1 mM ATP and 75 nM topoisomerase II. 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. 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:

S. aureus DNA Topoisomerase II (Gyrase) Assay Kit Plus-100	DSA100KSE
96-Well *S. aureus* Topo IV DNA Decatenation Assay Kit Plus	SDD96KE
Human Topoisomerase I, 10,000 Units	HTOPI-010
E. coli DNA Topoisomerase II (Gyrase) Assay Kit Plus-100	DSA100KE
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 gyrase ATPase assay Kit Plus	T2A-100KE
96-well Topoisomerase DNA Cleavage Assay Kit	TDC96K

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.