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INSTRUCTIONS

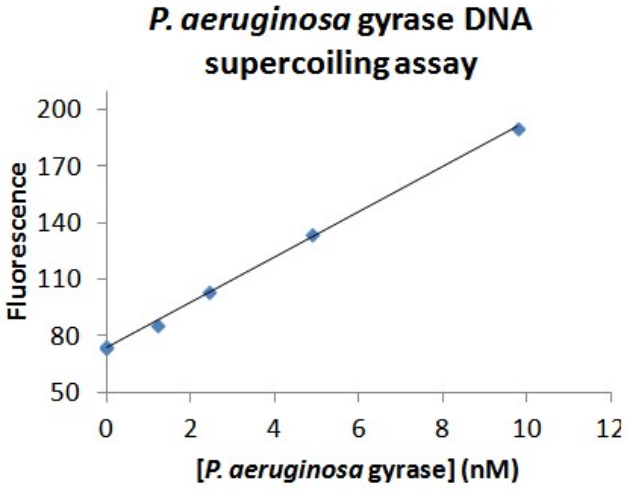
ProFoldin
P. aeruginosa DNA Gyrase DNA Supercoiling Assay Kits

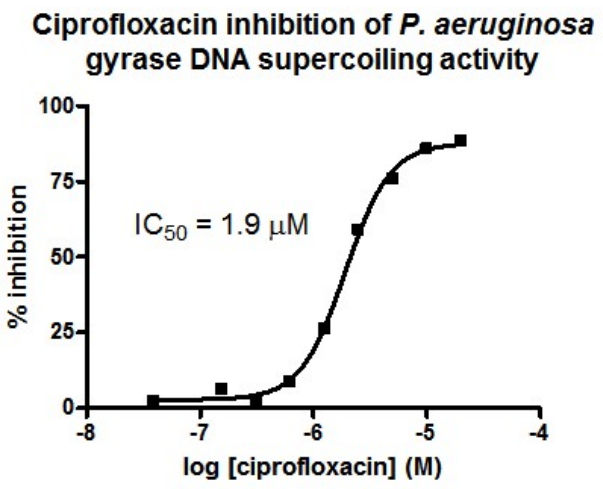
***P. aeruginosa* DNA Gyrase Assay Kit Plus-100** **Catalog No. DSA100KP**

H19 Dye for DNA Relaxation and Supercoiling Assays **Catalog No. DSA1000D**

Introduction

DNA topoisomerases such as bacterial topoisomerase II (gyrase) convert relaxed circular DNA into supercoiled DNA (DNA supercoiling reaction). The **DNA Topoisomerase II (Gyrase) Assay Kit** is based on the principle that the supercoiled DNA and relaxed DNA yield different fluorescent intensity when interact with fluorescence dye H19. The relaxed DNA suppresses the fluorescent intensity much more than the supercoiled DNA in the presence of magnesium. Therefore, when the relaxed DNA is converted into its supercoiled form, the fluorescent signal increases. The change of fluorescence intensity is used to measure the supercoiling reaction of gyrases and high throughput screen of gyrase inhibitors.





The ***P. aeruginosa* DNA Topoisomerase II (Gyrase) Assay Kit Plus-100** (Catalog No. DSA100KP) includes all the reagents for 100 assays of *P. aeruginosa* gyrase DNA supercoiling activity. It includes 600 µl of 10 x Buffer T2, 405 µl of 10 x relaxed DNA, 20 µl of 1500 x H19 dye, 450 µl of 10 x ATP, 3000 µl of 10 x H19 dilution buffer and 50 µl 100 x *P. aeruginosa* gyrase.

The **H19 Dye for DNA Relaxation and Supercoiling Assays** (Catalog No. DSA1000D) includes 170 µl of 1500 x Dye H19 and 26 ml of 10 x H19 dilution buffer. It is for 1000 assays of DNA relaxation or supercoiling reactions in a 96-well plate format.

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Assay Protocol

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.

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:

E. coli DNA Gyrase Assay Kit Plus-100	Catalog No. DSA100KE
S. aureus DNA Gyrase Assay Kit Plus-100	Catalog No. DSA100KSE
Human Topoisomerase II DNA Decatenation Assay Kit Plus-100	Catalog No. HDC100KE
Human Topoisomerase I DNA Relaxation Assay Kit Plus-100	Catalog No. HRA100KE
96-Well E. coli Topoisomerase I DNA Decatenation Assay Plus	Catalog No. T1DD96KE
96-Well E. coli Topo IV DNA Decatenation Assay Plus	Catalog No. EDD96KE

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.