



ProFoldin

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INSTRUCTIONS

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S. aureus DNA Gyrase DNA Supercoiling Assay Kits

S. aureus DNA Topoisomerase II (Gyrase) Assay Kit Plus Catalog No. DSA020KSE

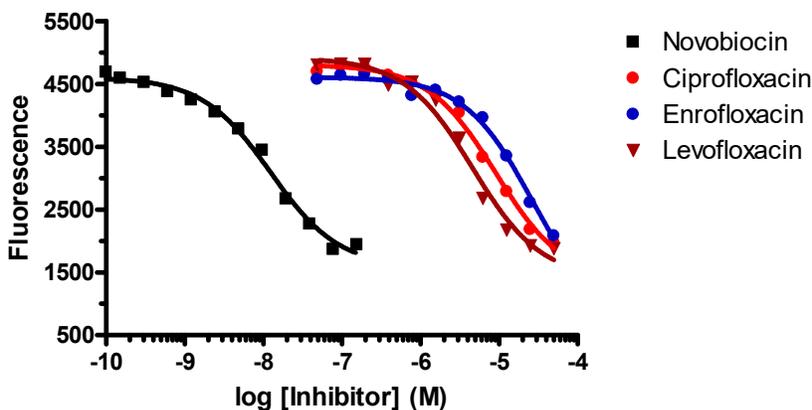
S. aureus DNA Topoisomerase II (Gyrase) Assay Kit Plus-100 Catalog No. DSA100KSE

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.

Inhibition of *S. aureus* gyrase DNA supercoiling activity



The *S. aureus* DNA Topoisomerase II (Gyrase) Assay Kit Plus (Catalog No. DSA020KSE) includes 160 μ l of 10 x Buffer T2, 84 μ l of 10 x relaxed DNA, 6 μ l of 1500 x H19 dye, 90 μ l of 10 x ATP, 10 μ l 7.5 μ M *S. aureus* gyrase, 180 μ l of 2 M potassium glutamate and 550 μ l of 10 x H19 dilution buffer. It is for 20 assays in a 96-well plate format.

The *S. aureus* DNA Topoisomerase II (Gyrase) Assay Kit Plus-100 (Catalog No. DSA100KSE) includes 500 μ l of 10 x Buffer T2, 410 μ l of 10 x relaxed DNA, 22 μ l of 1500 x H19 dye, 450 μ l of 10 x ATP, 43 μ l 7.5 μ M *S. aureus* gyrase, 820 μ l of 2 M potassium glutamate and 3000 μ l of 10 x H19 dilution buffer. It is for 100 assays in a 96-well plate format.



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The **H19 Dye for DNA Relaxation and Supercoiling Assays** (Catalog No. DSA1000D) includes 170 μ l 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.

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 μ l including: 19.6 μ l of H₂O, 4 μ l of 10 x buffer, 8 μ l of 2 M potassium glutamate, 4 μ l of 10 x relaxed DNA (250 μ g/ml), 0.4 μ l of 7.5 μ M *S. aureus* gyrase, 4 μ l 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 NH₄OAc, 4.6 % glycerol, 1 mM DTT, 0.005% Brij35, 8 mM MgCl₂, 400 mM potassium glutamate, 25 μ g/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 μ l 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 μ l of the freshly prepared H19 dye with each reaction solution (40 μ l). 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:

<i>E.coli</i> Gyrase DNA Supercoiling Assay Kit Plus-100	Catalog No. DSA100KE
<i>P. aeruginosa</i> Gyrase DNA Supercoiling Assay Plus-100	Catalog No. DSA100KP
Human Topoisomerase II DNA Decatenation Assay Kit Plus-100	Catalog No. HDC100KE
DNA Topoisomerase I Assay Kit Plus-100	Catalog No. DRA100KE
Human topoisomerase I DNA relaxation assay kit plus-100	Catalog No. HRA100KE
Human topoisomerase I, 10,000 Units	Catalog No. HTOPI-010
Relaxed plasmid DNA - 0.050 mg	Catalog No. RDNA-050UG
Supercoiled plasmid DNA - 0.050 mg	Catalog No. SDNA-050UG

For more information of DNA topoisomerase assays, please see the website of The Topo World: http://www.profoldin.com/topoisomerase_assays_1.html. For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.