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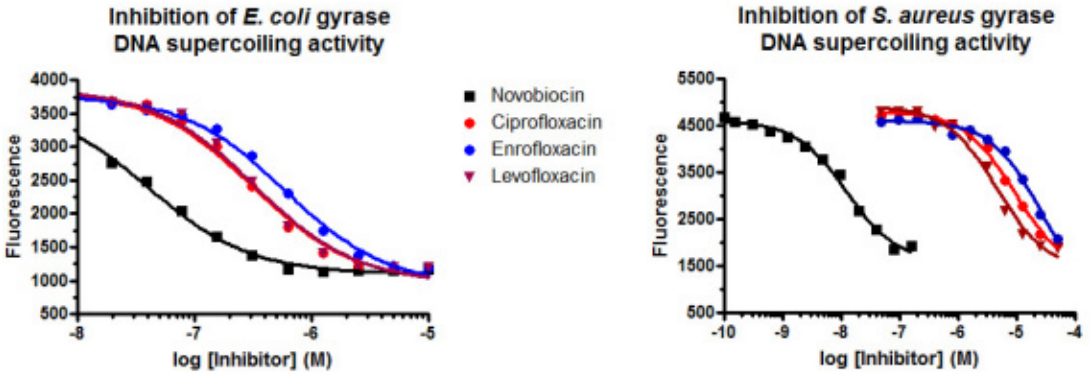
INSTRUCTIONS

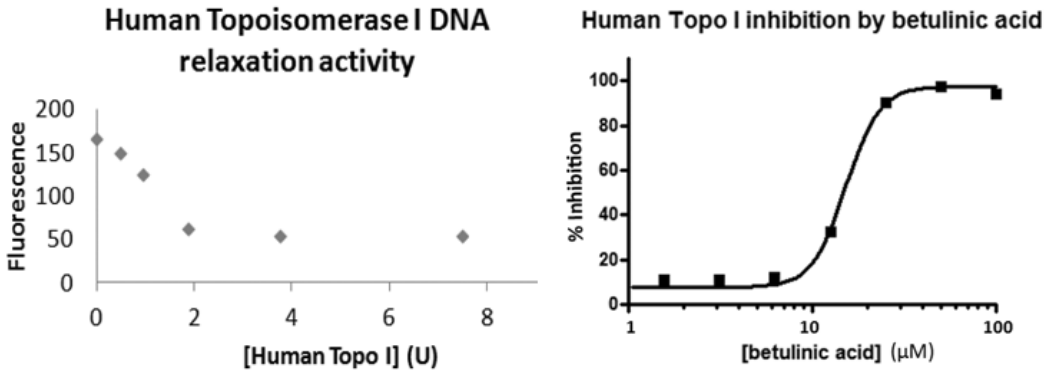
ProFoldin
H19 Dye for DNA Supercoiling and Relaxation Assays

H19 Dye for DNA Relaxation and Supercoiling Assays **(Cat. No.: DSA1000D)**
H19 Dye for DNA Relaxation and Supercoiling Assays –HTS package 1 **(Cat. No.: DSA2500D)**
10x H19 dilution buffer **(Cat. No.: H19BUF-10)**

Introduction

H19 dye is a fluorescence dye specifically designed for high throughput DNA supercoiling and relaxation assays. DNA topoisomerases such as bacterial gyrases convert relaxed circular DNA into supercoiled DNA (DNA supercoiling reaction). The reverse reaction that converts supercoiled DNA to relaxed DNA (DNA relaxation reaction) is carried out by DNA topoisomerase I. The assay principle is based on 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. When the supercoiled DNA is converted into its relaxed form, the fluorescent signal decreases. The difference of fluorescence intensity is used to measure the DNA topology changes by various DNA topoisomerases and high throughput screen of topoisomerase inhibitors. The assay is in a 96-well plate format. It can be modified to 384-well plate format.





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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.

The **H19 Dye for DNA Relaxation and Supercoiling Assays –HTS package 1** (Catalog. No.DSA2500D) includes 425 µl of 1500 x Dye H19 and 65 ml of 10 x H19 dilution buffer. It is for 2500 assays of DNA relaxation or supercoiling reactions in a 96-well plate format.

The **10x H19 dilution buffer** (Catalog No. H19BUF-10) includes 100 ml of 10 x H19 dilution buffer.

Assay Protocol for *E. coli* gyrase DNA supercoiling assay

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 *E. coli* gyrase. 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 Topoisomerase II (Gyrase) Assay Kit Plus-100	Catalog No. DSA100KE
Human topoisomerase I DNA relaxation assay kit plus-100	Catalog No. HRA100KE
Human topoisomerase I, 10,000 Units	Catalog No. HTOPI-010
Human Topoisomerase I, 100,000 units	Catalog No. HTOPI-100
Relaxed Plasmid DNA (1 mg)	Catalog No. RDNA-1MG
Supercoiled plasmid DNA (1mg)	Catalog No. SDNA-1 MG

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