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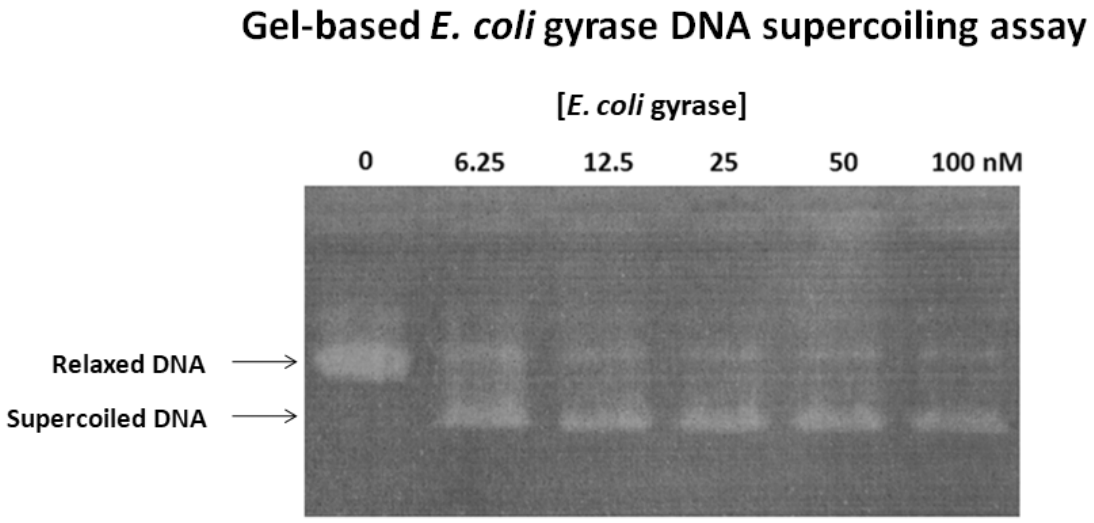
INSTRUCTIONS

ProFoldin
Gel-Based *E. coli* Topoisomerase II (Gyrase) DNA Supercoiling Assay Kit

Catalog Number: GDSA100KE

Introduction

DNA topoisomerases such as bacterial topoisomerase II (gyrase) convert relaxed circular DNA into supercoiled DNA (DNA supercoiling reaction). The **Gel-Based *E. coli* Topoisomerase II (Gyrase) DNA Supercoiling Assay Kit** is based on the principle that the supercoiled DNA and relaxed DNA are separated by agarose gel electrophoresis. Fluorescence-based DNA supercoiling assays in a 96-well pate format are also available for high throughput screening of gyrase inhibitors. For more information of the high throughput gyrase DNA supercoiling assays, please visit the website at http://www.profoldin.com/topoisomerase_assays_1.html.



The **Gel-Based *E. coli* DNA Topoisomerase II (Gyrase) Assay Kit Plus-100** (Catalog No. GDSA100KE) includes all the reagents for 100 samples in gel-based assays of *E. coli* gyrase DNA supercoiling activity. It includes 400 µl of 10 x Buffer, 105 µl of 250 µg/ml relaxed DNA, 220 µl of 10 mM ATP, 550 µl of 5 x Gel loading buffer and 25 µl of 10 µM *E.coli* Topoisomerase IV (100 x).

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Assay Protocol

1. Reaction:

The total volume of each reaction mixture is 20 µl including: 13 µl of H_2O, 2 µl of 10 x Buffer, 1 µl of 250 µg/ml relaxed DNA, 2 µl of 200 nM *E. coli* gyrase (10 x), 2 µl of 10 mM ATP. Incubate the reaction mixture at 37°C for 60 min. At the end of the reaction, add 5 µl of 5 x gel loading buffer.

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 $MgCl_2$, 12.5 µg/ml relaxed plasmid DNA, 1 mM ATP and 20 nM topoisomerase II. The 10 x enzyme is prepared by dilution of the 100 x enzyme in 1x assay buffer. A negative control reaction can be the reaction mixture without addition of ATP.

2. Agarose gel electrophoresis

(1) Prepare 1 % agarose gel in 1 x TAE buffer.
(2) Load 25 ul of the sample.
(3) Run the gel at 100 V for 90 min to 2 hours.
(4) Stain the gel in an ethidium bromide solution and destain the gel in water.
(5) Take a picture of the gel under UV light.

Related Products:

E. coli DNA Topoisomerase II (Gyrase) Assay Kit Plus-100	Catalog No. DSA100KE
S. aureus Gyrase DNA Supercoiling Assay Kit Plus-100	Catalog No. DSA100KSE
E. coli DNA Topoisomerase I Assay Kit Plus-100	Catalog No. DRA100KE
Human Topoisomerase I DNA Relaxation Assay Kit Plus -100	Catalog No. HRA100KE
Human Topoisomerase II DNA Decatenation Assay Kit Plus-100	Catalog No. HDC100KE

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.