

**ProFoldin**

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INSTRUCTIONS

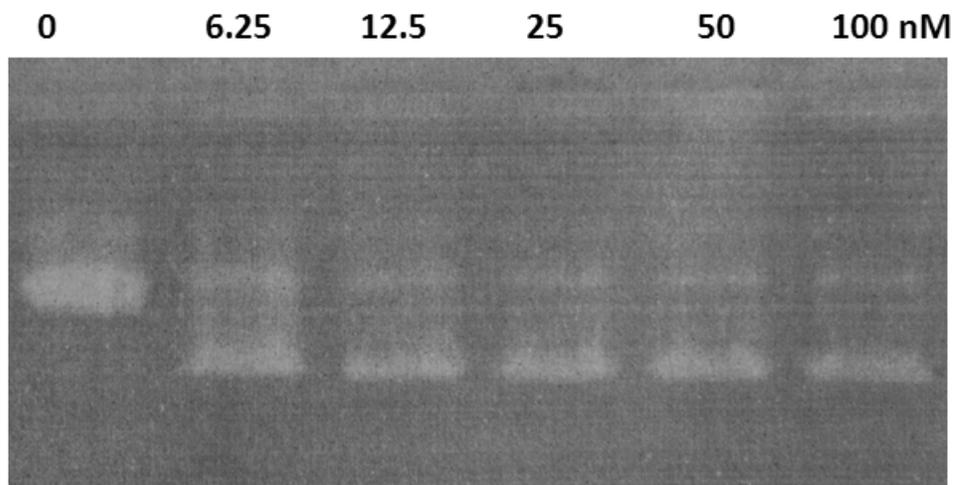
ProFoldin Gel-Based *E. coli* Topoisomerase II (Gyrase) DNA Supercoiling Assay Kit

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 plate 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.

E. coli gyrase DNA supercoiling assay – enzyme dose response



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 μ l of 10 x Buffer, 105 μ l of 250 μ g/ml relaxed DNA, 220 μ l of 10 mM ATP, 550 μ l of 5 x Gel loading buffer and 25 μ l of 2000 nM *E. coli* gyrase (100 x).

The **Gel-Based *E. coli* DNA Topoisomerase II (Gyrase) Assay Kit Plus-50** (Catalog No. GDSA050KE) includes the half volume of the GDSA100KE kit.



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Assay Protocol

1. Reaction:

The total volume of each reaction mixture is 20 μ l including: 13 μ l of H₂O, 2 μ l of 10 x Buffer, 1 μ l of 250 μ g/ml relaxed DNA, 2 μ l of 200 nM *E. coli* gyrase (10 x), 2 μ l of 10 mM ATP. Incubate the reaction mixture at 37°C for 60 min. At the end of the reaction, add 5 μ l of 5 x gel loading buffer.

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, 12.5 μ g/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 μ l 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:

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

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.