



ProFoldin

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INSTRUCTIONS

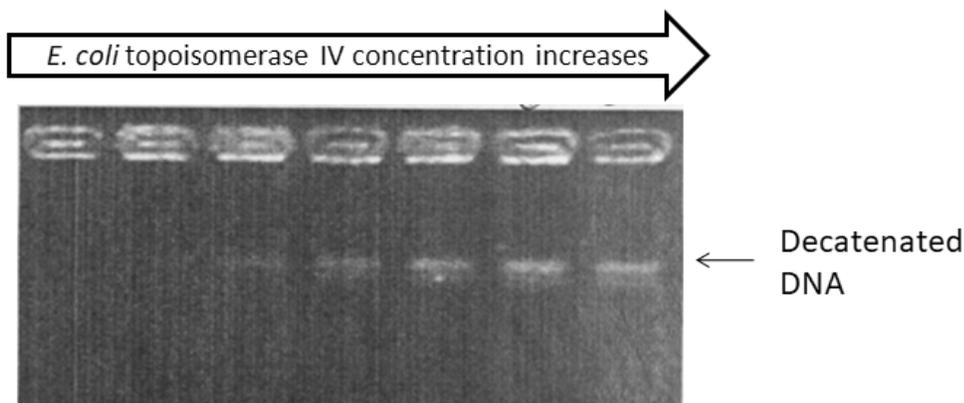
ProFoldin Gel-Based *E. coli* Topoisomerase IV DNA Decatenation Assay Kit Plus

Catalog Number: GDDA100KE

Introduction

DNA decatenation is an essential process during DNA replication in the cells. The DNA decatenation reaction was carried out by topoisomerase IV (the parC-parE complex) in bacteria. The DNA decatenation reaction converts the concatenated DNA into decatenated DNA. The **Gel-Based *E. coli* Topoisomerase IV DNA Decatenation Assay** is based on the principle that the decatenated DNA and concatenated DNA are separated by agarose gel electrophoresis. Fluorescence-based DNA decatenation assays in a 96-well plate format are also available for high throughput screening of gyrase inhibitors. For more information of the high throughput DNA decatenation assays, please visit the website at http://www.profoldin.com/topo_iv_assays.html.

Gel-based *E. coli* Topoisomerase IV DNA decatenation assay



The **Gel-Based *E. coli* Topoisomerase IV DNA Decatenation Assay Kit Plus** (Catalog No. GDDA100KE) includes all the reagents for 100 samples in gel-based assays of *E. coli* topoisomerase IV DNA decatenation activity. It includes 300 μl of 10 x Buffer, 205 μl of 60 $\mu\text{g/ml}$ concatenated DNA (kDNA), 205 μl of 10 mM ATP, 550 μl of 5 x Gel loading buffer and 22 μl of 10 μM *E. coli* topoisomerase IV (the parC-parE complex).

Assay Protocol

1. Reaction:

The total volume of each reaction mixture is 20 μl including: 13.8 μl of H_2O , 2 μl of 10 x assay buffer, 2 μl of 60 $\mu\text{g/ml}$ concatenated DNA, 2 μl of 10 mM ATP, 0.2 μl of 10 μM topoisomerase. 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.



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Note: The final concentrations for bacterial topoisomerase DNA decatenation assays are 20 mM Tris-HCl, pH 8, 35 mM NH₄OAc, 4.6 % glycerol, 1 mM DTT, 0.005% Brij35, 8 mM MgCl₂, 6 µg/ml concatenated DNA, 1 mM ATP and 10 nM *E. coli* topoisomerase IV. A negative control reaction can be the reaction mixture without enzyme or 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:

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| Supercoiled Plasmid DNA -1 mg | Catalog No. SDNA-1MG |
| Relaxed Plasmid DNA -1 mg | Catalog No. RDNA-1MG |
| 96-Well <i>E. coli</i> Topo IV DNA Decatenation Assay Plus | Catalog No. EDD96KE |
| 96-Well <i>S. aureus</i> Topo IV DNA Decatenation Assay Kit Plus | Catalog No. SDD96KE |
| 96-Well <i>S. pneumoniae</i> Topo IV DNA Decatenation Assay Kit Plus | Catalog No. NDD96KE |
| 96-Well <i>P. aeruginosa</i> Topo IV DNA Decatenation Assay Kit Plus | Catalog No. PDD96KE |
| Spin-column <i>E. coli</i> DNA Topoisomerase IV Assay Kit Plus | Catalog No. DDC020KE |
| 96-Well Human Topo II DNA Decatenation Assay Kit Plus | Catalog No. HDD96KE |
| <i>E. coli</i> DNA Topoisomerase I Assay Kit Plus | Catalog No. DRA020KE |
| <i>E. coli</i> DNA Topoisomerase I Assay Kit Plus-100 | Catalog No. DRA100KE |
| 96-Well <i>E. coli</i> Topoisomerase I DNA Decatenation Assay Plus | Catalog No. T1DD96KE |
| Gel-Based Human Topoisomerase I DNA relaxation Assay Kit | Catalog No. GHRA100KE |
| Gel-Based <i>E. coli</i> Topoisomerase I DNA relaxation Assay Kit | Catalog No. GDRA100KE |
| Human Topoisomerase I, 10,000 Units | Catalog No. HTOPI-010 |
| Human Topoisomerase I, 40,000 Units | Catalog No. HTOPI-040 |
| Human Topoisomerase I, 100,000 Units | Catalog No. HTOPI-100 |
| H19 Dye for DNA Relaxation and Supercoiling Assays | Catalog No. DSA1000D |
| Human DNA Topoisomerase I Assay Kit-100 | Catalog No. HRA100K |
| Human DNA Topoisomerase I Assay Kit Plus-100 | Catalog No. HRA100KE |
| Human DNA Topoisomerase I Assay Kit Plus-1000 | Catalog No. HRA1000KE |
| <i>E. coli</i> DNA Gyrase Assay Kit Plus-100 | Catalog No. DSA100KE |
| <i>S. aureus</i> DNA Gyrase Assay Kit Plus-100 | Catalog No. DSA100KSE |
| <i>P. aeruginosa</i> DNA Gyrase Assay Kit Plus-100 | Catalog No. DSA100KP |
| Gel-Based <i>E. coli</i> Gyrase DNA Supercoiling Assay Kit Plus-100 | Catalog No. GDSA100KE |

For more information of DNA topoisomerase assays, please see 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.