



## ProFoldin

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# INSTRUCTIONS

## ProFoldin

### 96-well Topoisomerase DNA Cleavage Assay Kits

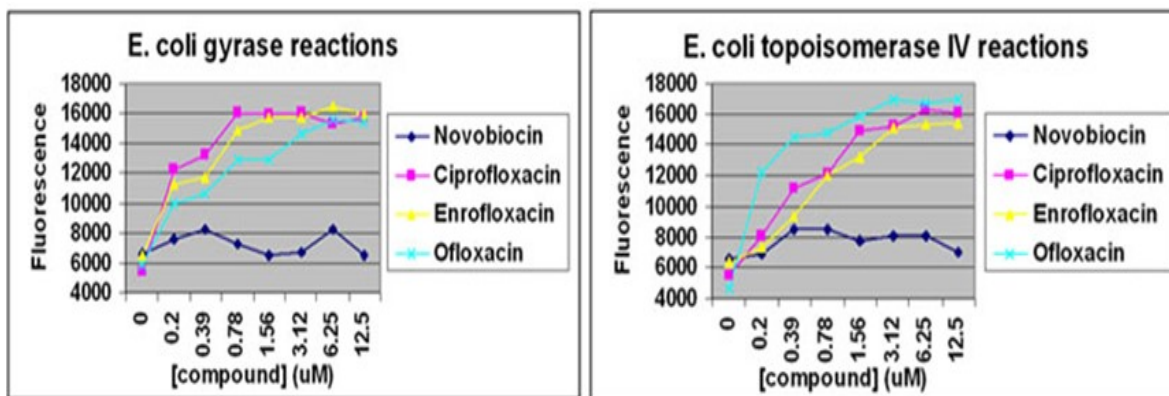
96-well Topoisomerase DNA Cleavage Assay Kit  
96-well *E. coli* Gyrase DNA Cleavage Assay Kit Plus  
96-well *E. coli* Topo IV DNA Cleavage Assay Kit Plus  
96-well *S. aureus* Topo IV DNA Cleavage Assay Kit Plus

Catalog No. TDC96K  
Catalog No. T2C96KE  
Catalog No. T4C96KE  
Catalog No. T4C96KS

### Introduction

Bacterial gyrase, topoisomerase IV (parC-parE) and mammalian topoisomerase II are type II topoisomerases. These topoisomerases change DNA topology by cleavage and religation of both DNA strands. When an inhibitor such as a fluoroquinolone drug blocks the religation step of the topoisomerase reaction, the covalent complex between the cleaved or linearized DNA and the topoisomerase (cleavage complex) is accumulated. Formation of the DNA cleavage complex causes DNA damage in the cells (topoisomerase poison). Testing formation of DNA cleavage complexes is useful for understanding the topoisomerase inhibition mechanism of novel topoisomerase inhibitors.

The 96-well topoisomerase DNA cleavage assay kits are based on the principle that the linearized DNA has much greater permeability through a solution matrix and a filter than the closed circular DNA. For example, both novobiocin and ciprofloxacin inhibit DNA gyrase. However, novobiocin does not cause formation of cleavage complex. Ciprofloxacin does. The assay kit is in 96-well plate format and can be used for testing topoisomerase poison compounds in a high throughput setting.



The **96-well Topoisomerase DNA Cleavage Assay Kit (Catalog No. TDC96K)** includes 300  $\mu$ l of 10 x reaction buffer, 30  $\mu$ l of 10 x DNA, 260  $\mu$ l of SDS solution, 30  $\mu$ l of 100 mM ATP, 260  $\mu$ l of proteinase K solutions, 10 ml of TDC matrix, 6 ml of rinse buffer, 500  $\mu$ l of 10 x dye, a black receiver plate, a V-bottom reaction plate and a TDC filter plate. Topoisomerase is not included in the kit. A plate filtration device (see Note below) and a vacuum line are needed for the plate filtration process.

The **96-well *E. coli* Gyrase DNA Cleavage Assay Kit Plus (Catalog No. T2C96KE)** includes all the components in TDC96K plus 26  $\mu$ l of 100 x *E. coli* Gyrase.



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The **96-well *E. coli* Topo IV DNA Cleavage Assay Kit Plus (Catalog No. T4C96KE)** includes all the components in TDC96K plus 26 µl of 100 x *E. coli* Topo IV.

The **96-well *S. aureus* Topo IV DNA Cleavage Assay Kit Plus (Catalog No. T4C96KS)** includes all the components in TDC96K plus 26 µl of 100 x *S. aureus* Topo IV.

## Equipment required (not provided with the kits)

A lab vacuum system: A lab vacuum line or pump (vacuum up to 80 kpa or 600 mmHg).  
A vacuum device: A plate vacuum device: Pall Corporation, Catalog No. 5017.  
A fluorescence reader: A plate fluorescence reader with excitation at 485 nm and emission at 535 nm.

**Storage:** Store the TDC filter plate and the TDC matrix at 4°C and the other reagents at -20 °C

## Assay Protocol

### 1. Reaction and sample preparation:

The total volume of each reaction mixture is 25 µl including: 0.5 µl of inhibitor, 19 µl of H<sub>2</sub>O, 2.5 µl of 10 x TDC buffer, 0.25 µl of 100 x DNA, 0.25 µl of 0.1 M ATP, 2.5 µl of 1 µM topoisomerase. Incubate the reaction mixture at 37°C for 60 min. Add 2.5 µl of SDS and mix the solution. Then add 2.5 µl of proteinase K and mix the solution and incubate the solution at 37°C for 60 min. Add 100 µl of the TDC matrix and mix for 5 min.

### 2. Assay

Place a 96-well black plate in the filtration device as a receiver of the filtration. Place the TDC filter plate on the top of the device. Load 110 µl of the sample onto the filter. Apply the vacuum (80 kpa or 600 mmHg) until the solution goes through the filter (about 5 min). Add 50 µl of the Rinse buffer and let the buffer completely go through the filter. Stop the vacuum and take out the receiver plate. Add 50 µl of the 1 x dye (diluted from the 10 x dye with water) into each well. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

## 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
96-Well <i>E. coli</i> Topo IV DNA Decatenation Assay Kit Plus	Catalog No. EDD96KE
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

For more information of topoisomerase and other drug target assays, please visit [www.profoldin.com](http://www.profoldin.com).