



**ProFoldin**

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## INSTRUCTIONS

# ProFoldin Quinolone-resistant *E. coli* Gyrase Mutant DNA Supercoiling Assay Kits

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Quinolone-resistant *E. coli* Gyrase Mutant S83W Assay Kit Plus-100

Cat#: S83W100KE

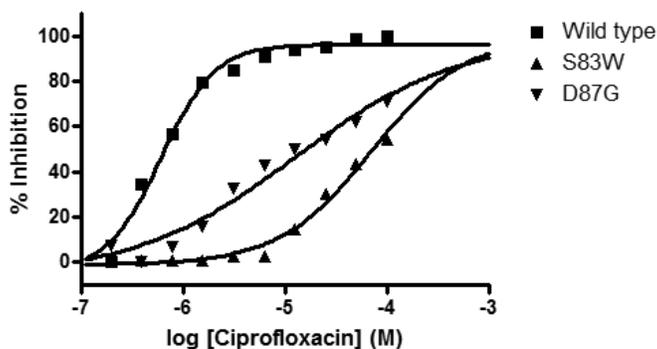
Quinolone-resistant *E. coli* Gyrase Mutant D87G Assay Kit Plus-100

Cat#: D87G100KE

### Introduction

Quinolone molecules such as ciprofloxacin are commonly used antibiotics that target bacterial DNA topoisomerases. Mutations S83W and D87G in the *gyrA* subunit of *E. coli* gyrase are proven causing quinolone resistance. Solving the drug resistance problem requires discovery of new molecules that inhibit the mutant topoisomerases. The **Quinolone-resistant *E. coli* Gyrase Mutant DNA Supercoiling Assay Kits** are designed to measure the inhibitory activities of molecules against mutant bacterial gyrases. The assays can be used to test new quinolone compounds in drug discovery.

Wild-type and mutant *E. coli* gyrase inhibition by ciprofloxacin



The **Quinolone-resistant *E. coli* Gyrase Mutant S83W Assay Kit Plus-100** (Catalog number S83W100KE) includes all the reagents for 100 assays of *E. coli* gyrase mutant S83W DNA supercoiling activity. It includes 600  $\mu$ l of 10 x Buffer T2, 405  $\mu$ l of 10 x relaxed DNA, 20  $\mu$ l of 1500 x H19 dye, 450  $\mu$ l of 10 x ATP, 3000  $\mu$ l of 10 x H19 dilution buffer and 50  $\mu$ l 100 x *E. coli* gyrase mutant S83W.

The **Quinolone-resistant *E. coli* Gyrase Mutant D87G Assay Kit Plus-100** (Catalog number D87G100KE) includes all the reagents for 100 assays of *E. coli* gyrase mutant D87G DNA supercoiling activity. It includes 600  $\mu$ l of 10 x Buffer T2, 405  $\mu$ l of 10 x relaxed DNA, 20  $\mu$ l of 1500 x H19 dye, 450  $\mu$ l of 10 x ATP, 3000  $\mu$ l of 10 x H19 dilution buffer and 50  $\mu$ l 100 x *E. coli* gyrase mutant D87G.

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# INSTRUCTIONS

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## Assay Protocol

The following assay protocol is based on a 96-well assay plate format using a standard black 96-well plate such as Greiner 655076.

### 1. Reaction:

The total volume of each reaction mixture is 40  $\mu$ l including: 24  $\mu$ l of H<sub>2</sub>O, 4  $\mu$ l of 10 x buffer, 4  $\mu$ l of 10 x relaxed DNA (250  $\mu$ g/ml relaxed plasmid DNA), 4  $\mu$ l of 10 x enzyme, 4  $\mu$ l 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<sub>4</sub>OAc, 4.6 % glycerol, 1 mM DTT, 0.005% Brij35, 8 mM MgCl<sub>2</sub>, 25  $\mu$ g/ml relaxed plasmid DNA, 1 mM ATP and 50 nM topoisomerase II. The 10 x enzyme is prepared by dilution of the 100x enzyme in 1x assay buffer. 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  $\mu$ l 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  $\mu$ l of the freshly prepared H19 dye with each reaction solution (40  $\mu$ l). 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:

Supercoiled Plasmid DNA -1 mg	Catalog No. SDNA-1MG
Relaxed Plasmid DNA -1 mg	Catalog No. RDNA-1MG
H19 Dye for DNA Relaxation and Supercoiling Assays	Catalog No. DSA1000D
<i>E. coli</i> DNA Topoisomerase I Assay Kit Plus-100	Catalog No. DRA100KE
Human Topoisomerase I, 10,000 Units	Catalog No. HTOPI-010
Human DNA Topoisomerase I Assay Kit Plus-100	Catalog No. HRA100KE
<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
Human Topoisomerase II DNA Decatenation Assay Kit Plus-100	Catalog No. HDC100KE
96-Well <i>E. coli</i> Topo IV DNA Decatenation Assay Plus	Catalog No. EDD96KE
<i>E. coli</i> gyrase ATPase assay Kit Plus	Catalog No. T2A-100KE
<i>E. coli</i> Topo IV ATPase Assay Kit Plus	Catalog No. T4A-100KE
96-well <i>E. coli</i> Gyrase DNA Cleavage Assay Kit Plus	Catalog No. T2C96KE
96-well <i>E. coli</i> Topo IV DNA Cleavage Assay Kit Plus	Catalog No. T4C96KE

For more information of DNA topoisomerase assays and assays for more drug targets and enzymes, please visit [www.profoldin.com](http://www.profoldin.com) or send emails to [info@profoldin.com](mailto:info@profoldin.com).