



**ProFoldin**

10 Technology Drive, Suite 40, Number 188

Hudson, MA 01749-2791 USA

Tel: (508) 735-2539 FAX: (508) 845-9258

[www.profoldin.com](http://www.profoldin.com)

[info@profoldin.com](mailto:info@profoldin.com)

## INSTRUCTIONS

# ProFoldin DNA Topoisomerase ATPase Assay Kits

*E. coli* gyrase ATPase assay Kit Plus

*S. aureus* gyrase ATPase assay Kit Plus

*E. coli* Topo IV ATPase Assay Kit Plus

*S. pneumoniae* Topo IV ATPase Assay Kit Plus

*P. aeruginosa* Topoisomerase IV ATPase Assay Kit Plus

*S. aureus* Topoisomerase IV ATPase Assay Kit Plus

Catalog No. T2A-100KE

Catalog No. T2A-100KS

Catalog No. T4A-100KE

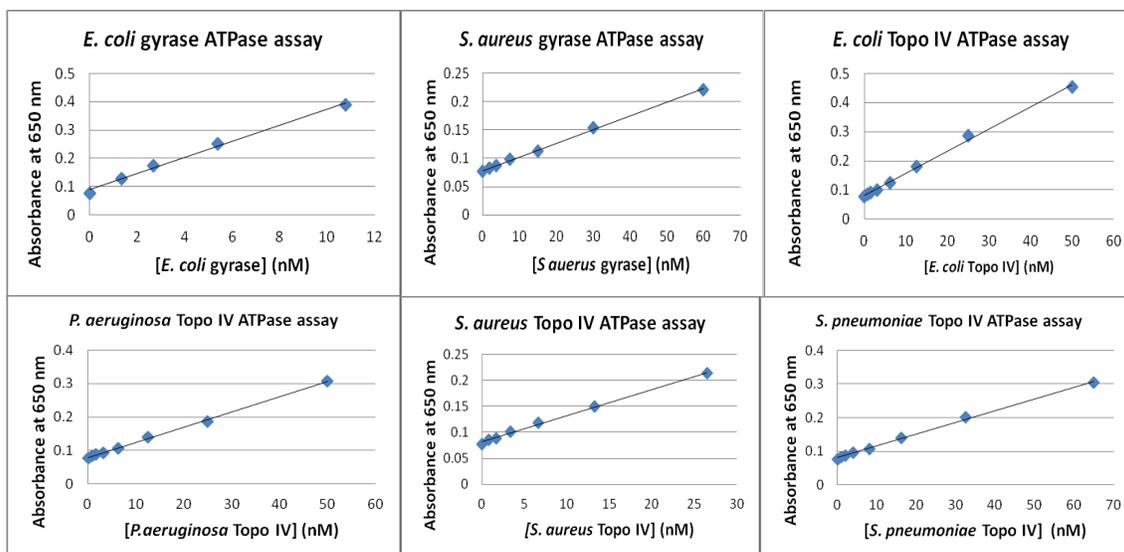
Catalog No. T4A-100KN

Catalog No. T4A-100KP

Catalog No. T4A-100KS

## Introduction

Type II DNA topoisomerases including topoisomerase II and topoisomerase IV hydrolyze ATP as the source of molecular energy to carry out DNA supercoiling and DNA decatenation reactions. Inhibition of the ATPase activity of topoisomerases by compounds such as novobiocin blocks their biological functions. Therefore, the topoisomerase ATPase assay can be used for high-throughput screen of topoisomerase inhibitors. The **Topoisomerase ATPase Assay Kit** is based on detection of the phosphate produced by the ATP hydrolysis reaction in the presence of DNA. The assay is in a 384-well plate format and the phosphate is detected using light absorbance at 650 nm.



The *E. coli* gyrase ATPase assay Kit Plus (Catalog No. T2A-100KE) includes 400  $\mu$ l of 10 x Buffer, 35  $\mu$ l of 100 x DNA, 33  $\mu$ l of 100x *E. coli* gyrase, 35  $\mu$ l of 100 x ATP and 5 ml of Dye for 100 assays of *E. coli* gyrase ATPase activity in a 384-well assay format.

The *S. aureus* gyrase ATPase assay Kit Plus (Catalog No. T2A-100KS) includes 400  $\mu$ l of 10 x Buffer, 35  $\mu$ l of 100x DNA, 33  $\mu$ l of 100 x *S. aureus* gyrase, 35  $\mu$ l of 100 x ATP and 5 ml of Dye for 100 assays of *S. aureus* gyrase ATPase activity in a 384-well assay format.



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The ***E. coli* Topo IV ATPase Assay Kit Plus** (Catalog No. T4A-100KE) includes 400 µl of 10 x Buffer, 35 µl of 100 x DNA, 33 µl of 100 x *E. coli* Topo IV, 35 µl of 100 x ATP and 5 ml of Dye for 100 assays of *E. coli* Topo IV ATPase activity in a 384-well assay format.

The ***S. pneumoniae* Topo IV ATPase Assay Kit Plus** (Catalog No. T4A-100KN) includes 400 µl of 10 x Buffer, 35 µl of 100 x DNA, 33 µl of 100 x *S. pneumoniae* Topo IV, 35 µl of 100 x ATP and 5 ml of Dye for 100 assays of *S. pneumoniae* Topo IV ATPase activity in a 384-well assay format.

The ***P. aeruginosa* Topoisomerase IV ATPase Assay Kit Plus** (Catalog No. T4A-100KP) includes 400 µl of 10 x Buffer, 35 µl of 100 x DNA, 33 µl of 100 x *P. aeruginosa* Topo IV, 35 µl of 100 x ATP and 5 ml of Dye for 100 assays of *P. aeruginosa* Topo IV ATPase activity in a 384-well assay format.

The ***S. aureus* Topoisomerase IV ATPase Assay Kit Plus** (Catalog No. T4A-100KS) includes 400 µl of 10 x Buffer, 35 µl of 100 x DNA, 33 µl of 100 x *S. aureus* Topo IV, 35 µl of 100 x ATP and 5 ml of Dye for 100 assays of *S. aureus* Topo IV ATPase activity in a 384-well assay format.

## Assay Protocol

### 1. Reaction and sample preparation:

The total volume of each reaction mixture is 30 µl including: 18 µl of H<sub>2</sub>O, 3 µl of 10 x buffer, 3 µl of 10 x DNA, 3 µl of 10 x enzyme, 3 µl of 2 mM ATP. Incubate the reaction mixture at 37°C for 60 min.

Note: The 10 x DNA is prepared by 10-fold dilution of the 100 x DNA stock (1 mg/ml) in water. The 10 x enzyme is prepared by dilution of the 100 x enzyme stock in the 1x buffer. The 2 mM ATP is prepared by 50-fold dilution of the 100 mM ATP stock in water.

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>, 10 µg/ml DNA, 0.2 mM ATP and 50 nM topoisomerase. A negative control reaction can be the reaction mixture without addition of enzyme.

### 2. Assay

Add 45µl of Dye and read the light absorbance at 650 nm.

## Related Products:

For more information of DNA topoisomerase assays, including DNA relaxation, DNA supercoiling, DNA decatenation and DNA cleavage assays, please see website of The Topo World:

[http://www.profoldin.com/topoisomerase\\_assays\\_1.html](http://www.profoldin.com/topoisomerase_assays_1.html).

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