



ProFoldin

10 Technology Drive, Suite 40, Number 188

Hudson, MA 01749-2791 USA

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

www.profoldin.com

info@profoldin.com

INSTRUCTIONS

ProFoldin

Topoisomerase IV DNA Relaxation Assay Kits

E. coli Topo IV DNA Relaxation Assay Kit Plus

T4RA-100KE

S. pneumoniae Topo IV DNA Relaxation Assay Kit Plus

T4RA-100KN

P. aeruginosa Topo IV DNA Relaxation Assay Kit Plus

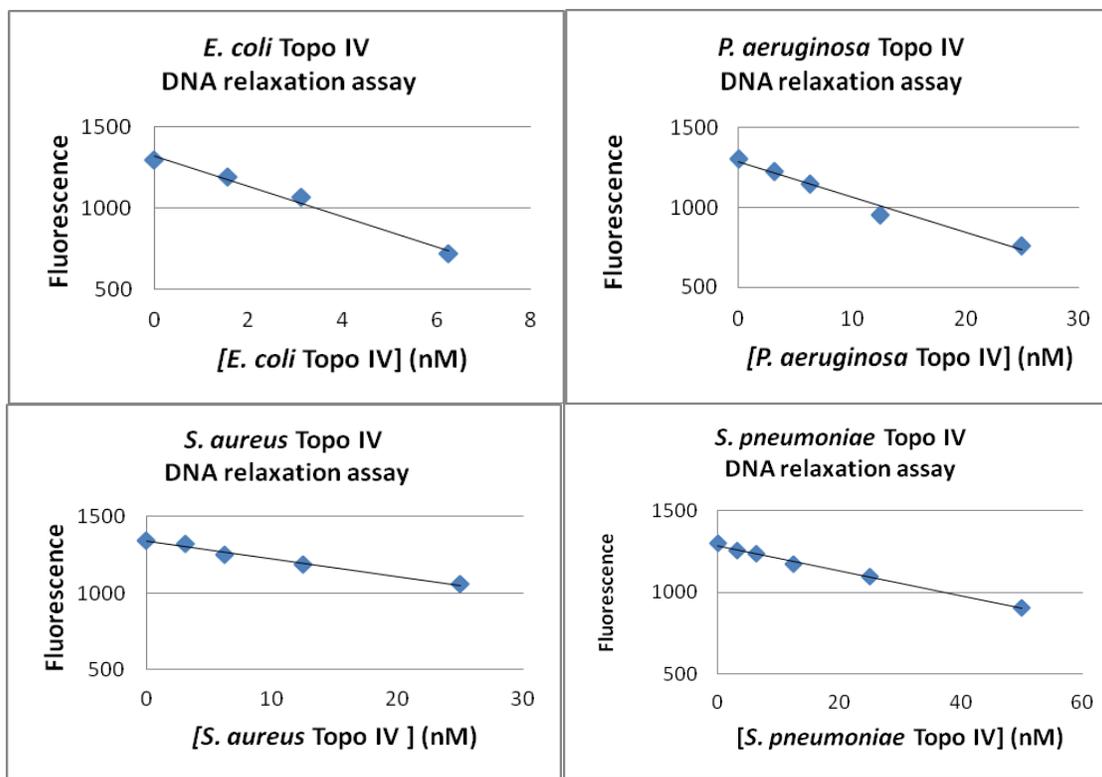
T4RA-100KP

S. aureus Topo IV DNA Relaxation Assay Kit Plus

T4RA-100KS

Introduction

DNA topoisomerase IV (the parC-parE complex) have DNA relaxation activity that converts the supercoiled DNA into its relaxed form. The Topoisomerase IV DNA Relaxation Assay Kit is based on the principle that the relaxed DNA suppresses the fluorescent intensity much more than the supercoiled DNA when the DNA interacts with fluorescence dye H19 (a fluorescence dye for DNA relaxation / supercoiling assay) in the presence of magnesium. When the supercoiled DNA is converted into its relaxed form, the fluorescent signal decreases.



The *E. coli* Topo IV DNA Relaxation Assay Kit Plus (T4RA-100KE) includes 480 μ l of 10 x Buffer, 405 μ l of 10 x supercoiled plasmid DNA, 45 μ l of 100 x *E. coli* topoisomerase IV, 420 μ l of 10



ProFoldin

10 Technology Drive, Suite 40, Number 188
Hudson, MA 01749-2791 USA
Tel: (508) 735-2539 FAX: (508) 845-9258
www.profoldin.com
info@profoldin.com

INSTRUCTIONS

mM ATP and 20 μ l of 1500 x fluorescence dye H19 for 100 assays of DNA relaxation reactions in a 96-well assay format.

The *S. pneumoniae* **Topo IV DNA Relaxation Assay Kit Plus** (T4RA-100KN) includes 480 μ l of 10 x Buffer, 405 μ l of 10 x supercoiled plasmid DNA, 45 μ l of 100 x *S. pneumoniae* topoisomerase IV, 420 μ l of 10 mM ATP and 20 μ l of 1500 x fluorescence dye H19 for 100 assays of DNA relaxation reactions in a 96-well assay format.

The *P. aeruginosa* **Topo IV DNA Relaxation Assay Kit Plus** (T4RA-100KP) includes 480 μ l of 10 x Buffer, 405 μ l of 10 x supercoiled plasmid DNA, 45 μ l of 100 x *P. aeruginosa* topoisomerase IV, 420 μ l of 10 mM ATP and 20 μ l of 1500 x fluorescence dye H19 for 100 assays of DNA relaxation reactions in a 96-well assay format.

The *S. aureus* **Topo IV DNA Relaxation Assay Kit Plus** (T4RA-100KS) includes 480 μ l of 10 x Buffer, 405 μ l of 10 x supercoiled plasmid DNA, 45 μ l of 100 x *S. aureus* topoisomerase IV, 420 μ l of 10 mM ATP and 20 μ l of 1500 x fluorescence dye H19 for 100 assays of DNA relaxation reactions in a 96-well assay format.

Assay Protocol

1. Reaction and sample preparation:

The total volume of each reaction mixture is 40 μ l including: 24 μ l of H₂O, 4 μ l of 10 x Buffer, 4 μ l of 10 x supercoiled DNA, 4 μ l of 10 mM ATP, 4 μ l of 1000 nM Topoisomerase VI. Incubate the reaction mixture at 37°C for 60 min.

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, 25 μ g/ml supercoiled plasmid DNA, 1 mM ATP and 100 nM topoisomerase IV.

2. Assay

- (1) Make 1x 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 μ l of the 1500 x H19 dye stock solution with 1500 μ l of 1x H19 Dilution Buffer (1500 x dilution). Mix the solution evenly by inverting the tube a few times.
- (2) Mix 250 μ l of the freshly prepared H19 dye with each reaction solution. Incubate the mixture for 5 min.
- (3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 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. For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.