



ProFoldin

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INSTRUCTIONS

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DNA Topoisomerase I Assay Kits

DNA Topoisomerase I Assay Kit

E. coli DNA Topoisomerase I Assay Kit Plus

E. coli DNA Topoisomerase I Assay Kit Plus-100

E. coli DNA Topoisomerase I Assay Kit Plus-1000

H19 Dye for DNA Relaxation and Supercoiling Assays

Catalog No. DRA020K

Catalog No. DRA020KE

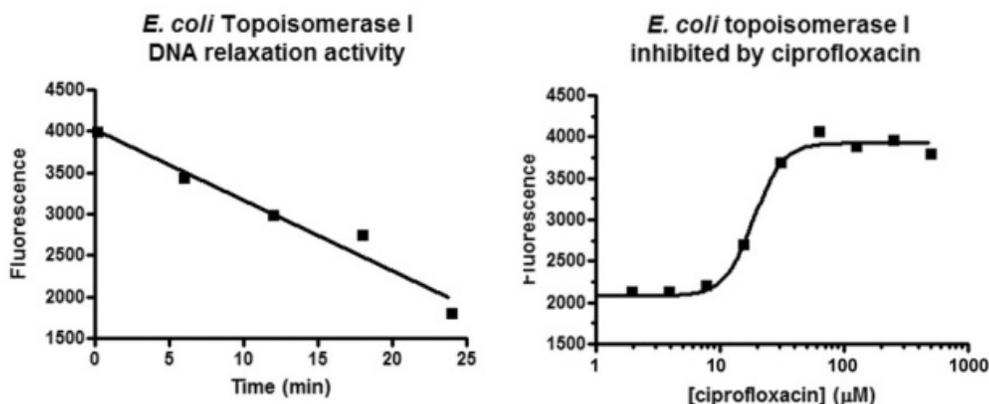
Catalog No. DRA100KE

Catalog No. DRA1000KE

Catalog No. DSA1000D

Introduction

DNA topoisomerases such as bacterial topoisomerase I convert supercoiled circular DNA into relaxed DNA (DNA relaxation reaction). The **DNA Topoisomerase I 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 **DNA Topoisomerase I Assay Kit** (Catalog number DRA020K) includes 160 μl of 10 x Buffer T1, 84 μl of 10 x supercoiled plasmid DNA, 8 μl of 1500 x Dye H19 and 500 μl of 10 x H19 dilution buffer. It is for 20 DNA relaxation assays in a 96-well plate format or 40 assays in a 384-well assay format.

The *E. coli* **DNA Topoisomerase I Assay Kit Plus** (Catalog No. DRA020KE) includes the kit reagents of DRA020K plus 10 μl of 100 x *E. coli* topoisomerase I.

The *E. coli* **DNA Topoisomerase I Assay Kit Plus-100** (Catalog No. DRA100KE) includes 480 μl of 10 x Buffer T1, 405 μl of 10 x supercoiled plasmid DNA, 20 μl of 1500 x Dye H19, 3000 μl of 10 x H19 dilution



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buffer and 42 μ l of 100 x *E. coli* topoisomerase I. It is for 100 DNA relaxation assays in a 96-well plate format or 200 assays in a 384-well assay format.

The **H19 Dye for DNA Relaxation and Supercoiling Assays** (Catalog No. DSA1000D) includes 170 μ l of 1500 x Dye H19 and 26 ml of 10 x H19 dilution buffer. It is for 1000 assays of DNA relaxation or supercoiling reactions in a 96-well plate format.

Assay Protocol

1. Reaction and sample preparation:

The total volume of each reaction mixture is 40 μ l including: 28 μ l of H₂O, 4 μ l of 10 x Buffer, 4 μ l of 10 x supercoiled DNA, 4 μ l of 10 x Topoisomerase I. The 10 x enzyme solution is freshly prepared by dilution of the enzyme stock solution with the 1 x Buffer. Incubate the reaction mixture at 37°C for 60 min.

Note: The final concentrations for the bacterial topoisomerase I assay are 16 mM Tris-HCl, pH 8, 6 mM MgCl₂, 25 μ g/ml supercoiled plasmid DNA and 40 nM topoisomerase I. Free magnesium is required for the H19 detection of the DNA topology. High concentrations of EDTA should be avoided in the reaction and detection solution.

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 the 1500x H19 dye stock solution with 1.5 ml 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 diluted 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:

Human topoisomerase I DNA relaxation assay kit plus-100	Catalog No. HRA100KE
Human topoisomerase I DNA relaxation assay kit plus-1000	Catalog No. HRA1000KE
DNA Topoisomerase II (Gyrase) Assay Kit Plus-100	Catalog No. DSA100KE
Human topoisomerase I, 40,000 Units	Catalog No. HTOPI-040
Human topoisomerase I, 100,000 Units	Catalog No. HTOPI-100

For more information of DNA topoisomerase assays including DNA supercoiling assays, DNA decatenation assays, topoisomerase DNA cleavage assays and DNA topoisomerase ATPase 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.