



ProFoldin

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INSTRUCTIONS

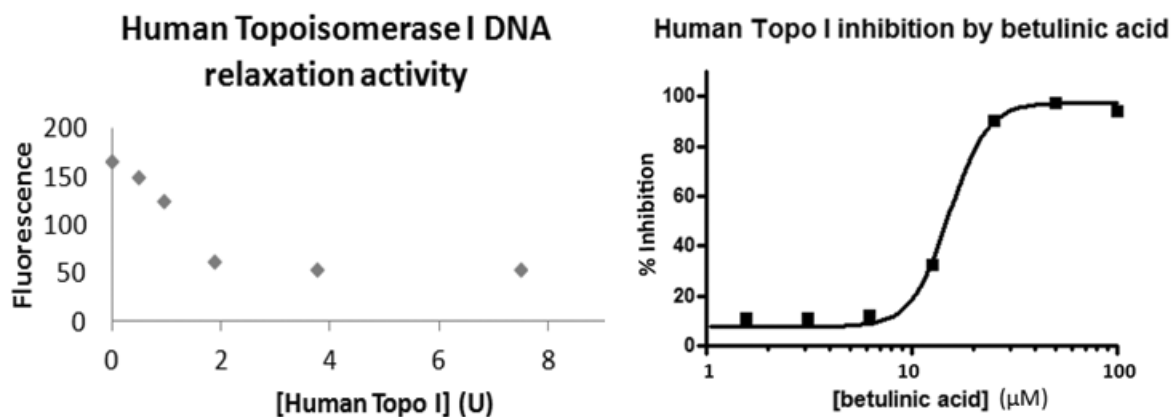
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Human DNA Topoisomerase I Assay Kits

Human Topoisomerase I DNA Relaxation Assay Kit	Catalog No. HRA020K
Human Topoisomerase I DNA Relaxation Assay Kit -100	Catalog No. HRA100K
Human Topoisomerase I DNA Relaxation Assay Kit Plus-100	Catalog No. HRA100KE
Human Topoisomerase I DNA Relaxation Assay Kit Plus-1000	Catalog No. HRA1000KE

Introduction

Human DNA topoisomerase I converts supercoiled circular DNA into relaxed DNA (DNA relaxation reaction). The **Human 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.



Human Topoisomerase I DNA Relaxation Assay Kit (Catalog No. HRA020K) includes 160 μ l of 10 x Buffer HT1, 84 μ l of 10 x supercoiled plasmid DNA, 8 μ l of 1500 x Dye H19 and 550 μ l 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. Human topo I enzyme is not included and can be purchased separately.

Human Topoisomerase I DNA Relaxation Assay Kit -100 (Catalog No. HRA100K) includes 480 μ l of 10 x Buffer HT1, 405 μ l of 10 x supercoiled plasmid DNA, 20 μ l of 1500 x Dye H19 and 3000 μ l of 10 x H19 dilution buffer. It is for 100 DNA relaxation assays in a 96-well plate format or 200 assays in a 384-well assay format. Human topo I enzyme is not included and can be purchased separately.

Human Topoisomerase I DNA Relaxation Assay Kit Plus-100 (Catalog No. HRA100KE) includes 480 μ l of 10 x Buffer HT1, 405 μ l of 10 x supercoiled plasmid DNA, 10 μ l of 500



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U/μl human topoisomerase I enzyme (1000 x), 20 μl of 1500 x Dye H19 and 3000 μl of 10 x H19 dilution buffer. It includes all the reagents for 100 DNA relaxation assays in a 96-well plate format or 200 assays in a 384-well assay format.

Human Topoisomerase I DNA Relaxation Assay Kit Plus-1000 (Catalog No. HRA1000KE)

includes 4800 μl of 10 x Buffer HT1, 4050 μl of 10 x supercoiled plasmid DNA, 45 μl of 500 U/μl human topoisomerase I enzyme (1000 x), 170 μl of 1500 x Dye H19 and 26 ml of 10 x H19 dilution buffer. It includes all the reagents for 1000 DNA relaxation assays in a 96-well plate format or 2000 assays in a 384-well assay format.

DNA Relaxation Assay Protocol

1. Reaction and sample preparation:

The total volume of each reaction mixture is 40 μl including: 28 μl of H₂O, 4 μl of 10 x Buffer HT1, 4 μl of 10 x supercoiled DNA (250 μg/ml), 4 μl of 10 x enzyme (1 U/μl). Incubate the reaction mixture at 37°C for 60 min.

Note: The final concentrations are 25 mM Tris-HCl, pH 7.4, 58 mM KCl, 0.25 mM DTT, 5 mM MgCl₂, 0.25 mM EDTA, 15 μg/ml BSA, 25 μg/ml supercoiled plasmid DNA and 0.1 U/μl topoisomerase I. A negative control reaction can be the reaction mixture without the enzyme.

2. Assay

- (1) For each 10 assays, freshly dilute 2 μl of 1500 x H19 dye into 3 ml of 1x H19 dilution buffer. The 1x H19 dilution buffer is prepared by 10 fold dilution of the 10x H19 dilution buffer with water. Mix the solution evenly by inverting the tube a few times.
- (2) Mix 250 μl of the freshly prepared 1 x H19 dye with each reaction solution (40 μl). Incubate the mixture at room temperature for 5 min.
- (3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Reference:

Abdellatif, Khaled R.A. et al, Design, Synthesis and Cytotoxicity Evaluation of New 3, 5-Disubstituted-2-Thioxoimidazolidinones. *Anti-Cancer Agents in Medicinal Chemistry*, Volume 18, 573 (2018).

Related Products:

Human Topoisomerase I, 10,000 Units	Catalog No. HTOPI-010
Human Topoisomerase I, 40,000 Units	Catalog No. HTOPI-040
Human Topoisomerase I, 100,000 Units	Catalog No. HTOPI-100
Gel-Based Human Topoisomerase I DNA relaxation Assay Kit	Catalog No. GHRA100KE
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

For more information of DNA topoisomerase 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.