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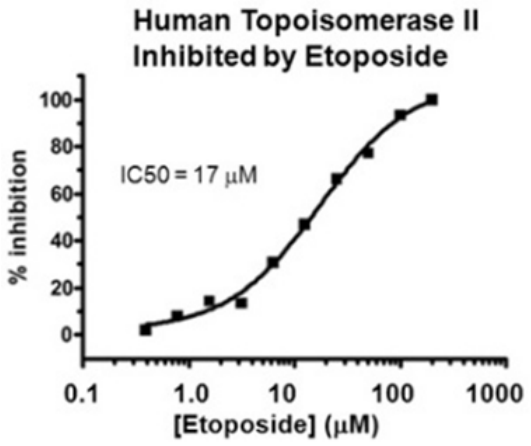
INSTRUCTIONS

ProFoldin
96-Well Human Topo II DNA Decatenation Assay Kits

96-Well Human Topo II DNA Decatenation Assay Kit Plus **Catalog No. HDD96KE**
96-Well Topoisomerase DNA Decatenation Assay Kit **Catalog No. TDD96K**

Introduction

DNA decatenation is an essential process during DNA replication in the cells. The DNA decatenation reaction was carried out by topoisomerase II in human. The DNA decatenation reaction converts the concatenated DNA into decatenated DNA. The **96-Well Topoisomerase DNA Decatenation Assay** is in a 96-well assay plate format that can be used for high-throughput tests of topoisomerase inhibitors. The assay is based on the principle that the decatenated DNA is separated from the concatenated DNA by a filtration process. The decatenated DNA passed through the filter (TDD filter plate), received in a black 96 well plate and quantified by fluorescence at 535 nm (excitation at 485 nm).



Each **96-Well Human Topo II DNA Decatenation Assay Kit Plus (Catalog No. HDD96KE)** includes 600 µl of 10 x assay buffer, 500 µl of 10 x concatenated DNA, 520 µl of 2 mM ATP, 26 µl of 200 x human Topo II, 500 µl of enzyme dilution buffer, 1 ml of Stop solution (0.4 M EDTA), 530 µl of 20 x fluorescence dye, 2 ml of 10 x rinse buffer, one V-bottom plate, a TDD filter plate and one black 96-well plates for 96 assays of DNA decatenation reactions.

Each **96-Well Topoisomerase DNA Decatenation Assay Kit (Catalog No. TDD96K)** includes 600 µl of 10 x assay buffer, 500 µl of 10 x concatenated DNA, 520 µl of 2 mM ATP, 600 µl of 0.4 M EDTA, 260 µl of 20 x fluorescence dye, 2 ml of 10 x rinse buffer, one V-bottom plate, a TDD filter plate and one black 96-well plates for 96 assays of DNA decatenation reactions.

Equipment required (not provided with the kits)

A lab vacuum system: A standard lab vacuum line or pump (vacuum up to 80 kpa or 600 mmHg).
A vacuum device: A plate vacuum device: Pall Corporation, Catalog No. 5017.

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Assay Protocol

1. Reagent preparation and filtration unit

Dilute the 20 x fluorescence dye 20-fold to make 1x fluorescence dye.
Dilute the 10 x Rinse buffer 10-fold with water to make 1 x Rinse buffer.
Assembly the filtration unit by connecting the filtration device to a vacuum line, placing the black 96-well plate in the chamber of the filtration device as a receiver of the filtration and the TDD filter plate on the top of the device.

2. Reaction and sample preparation:

The reactions are carried out in a V-bottom plate. The total volume of each reaction mixture is 50 µl. Step 1: mix 32.5 µl of H_2O, 5 µl of 10 x assay buffer, 5 µl of 10 x concatenated DNA and 5 µl of 2 mM ATP. Step 2, add 2.5 µl of 20 x topoisomerase (1 U/µl) which is freshly diluted from the 200 x stock solution 10 fold using the dilution buffer, Step 3, incubate the reaction mixture at 37°C for 60 min. At the end of the reaction, add 5 µl of Stop Solution to stop the reaction.

> *Note:* The final concentrations are 10 mM Tris-HCl, pH 8, 50 mM NaCl, 0.1 mM EDTA, 50 mM KCl, 5 mM $MgCl_2$, 15µg/ml BSA, 3 µg/ml concatenated DNA, 0.2 mM ATP and 50 U/ml human topoisomerase II alpha. A lower temperature will have a slower reaction. For IC50 experiments, enzyme dose response should be tested to avoid using too much enzyme. The enzyme must be diluted in the enzyme dilution buffer freshly. Do not store the diluted enzyme.

3. Assay

Load 50 µl of the sample onto the filter plate. Apply the vacuum (80 kpa or 600 mmHg) until the solution goes though the filter. Add 150 µl of the Rinse Buffer and let the buffer completely go through the filter. Stop the vacuum and take out the receiver plate. Add 50 µl of the 1 x dye into each well. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Publications

1. Abderrazzak Merzouki et al, Adva-27a, a Novel Podophyllotoxin Derivative Found to Be Effective against Multidrug Resistant Human Cancer Cells, Anticancer Research 32: 4423-4432 (2012).
2. Narayanan S. et al, A cell cycle-controlled redox switch regulates the topoisomerase IV activity. Genes Dev. 29(11):1175-87 (2015).

Related products

Human Topo II DNA Decatenation Assay Kit Plus-100 (spin-column format)	Catalog No. HDC100KE
96-Well *E. coli* Topo IV DNA Decatenation Assay Kit Plus	Catalog No. EDD96KE
Human Topoisomerase I, 10,000 Units	Catalog No. HTOPI-010
Human DNA Topoisomerase I Assay Kit Plus-100	Catalog No. HRA100KE
Human DNA Topoisomerase I Assay Kit Plus-1000	Catalog No. HRA1000KE
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 topoisomerase and other drug targets assays, please visit www.profoldin.com or send emails to info@profoldin.com.