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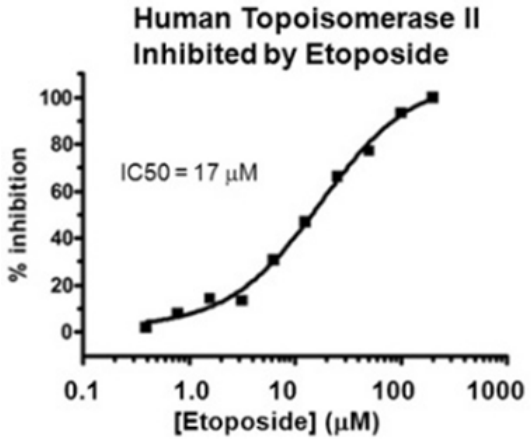
INSTRUCTIONS

ProFoldin
96-Well Human Topo II DNA Decatenation Assay Kits

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

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, 1200 µl of 50 mM $MgCl_2$, 510 µl of 10 x concatenated DNA (30 µg/ml kDNA), 520 µl of 10 x ATP (10 mM), 600 µl of 0.5 M EDTA, 275 µl of 20 x fluorescence dye, 50 µl of 10 U/µl human topoisomerase II alpha. 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. Reaction and sample preparation:

The total volume of each reaction mixture is 50 µl. The following protocol is for each 10 assay reactions. Step 1: Prepare a premix by mixing 247.5 µl of H_2O, 52.5 µl of 10 x assay buffer, 52.5 µl of 10 x kDNA, 52.5 µl of 10 mM ATP and 5 µl of 10 U/µl human Topo II enzyme. Step 2, add 39 µl of the premix into the wells containing 1 µl of inhibitor. Incubate the mixture for 5 min. Step 3: add 10 µl of 50 mM $MgCl_2$ to start the reaction and incubate the reaction mixture at 37°C for 60 min. Add 5 µl 0.5 M EDTA to stop the reaction.

2. 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.

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