



ProFoldin

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INSTRUCTIONS

ProFoldin Human Topoisomerase II DNA Decatenation Assay Kits

Human Topoisomerase II DNA Decatenation Assay Kit

Catalog No. HDC020K

Human Topoisomerase II DNA Decatenation Assay Kit Plus

Catalog No. HDC020KE

Human Topoisomerase II DNA Decatenation Assay Kit Plus-100

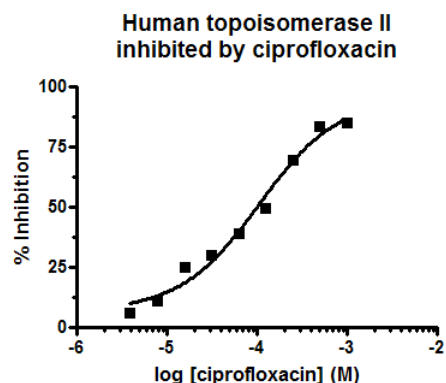
Catalog No. HDC100KE

DDC Spin-columns for DNA Decatenation Assays

Catalog No. DDC100

Introduction

Human DNA topoisomerase II converts the concatenated DNA into decatenated DNA (DNA decatenation reaction). The **Human Topoisomerase II DNA Decatenation Assay Kit** is based on the principle that the decatenated DNA is separated from the concatenated DNA by a quick and easy spin-column process. The concatenated DNA stays on the column, while the decatenated DNA is eluted. The eluted DNA is quantified by fluorescence.



The **Human Topoisomerase II DNA Decatenation Assay Kit** (Catalog number HDC020K) includes 125 μ l of 10 x assay buffer, 105 μ l of 10 x concatenated DNA (30 μ g/ml), 120 μ l of 10 x ATP (2 mM), 120 μ l of 0.5 M EDTA, 160 μ l of 20 x fluorescence dye and 20 spin columns for 20 assays of DNA decatenation reactions. Human topoisomerase II alpha is not included in the kit.

The **Human Topoisomerase II DNA Decatenation Assay Kit Plus** (Catalog number HDC020KE) includes all of the reagent in the **Human Topoisomerase II DNA Decatenation Assay Kit** (Catalog number HDC020K) plus 7 μ l of 200 x human topoisomerase II alpha (10 U/ μ l) and 0.2 ml of enzyme dilution buffer for 20 assays.

The **Human Topoisomerase II DNA Decatenation Assay Kit Plus -100** (Catalog number HDC100KE) includes 550 μ l of 10 x assay buffer, 520 μ l of 10 x concatenated DNA (30 μ g/ml), 550 μ l of 10 x ATP (2 mM), 600 μ l of 0.5 M EDTA, 800 μ l of 20 x fluorescence dye, 26 μ l of 200 x human topoisomerase II alpha (10 U/ μ l), 1 ml of enzyme dilution buffer and 100 spin columns for 100 assays of DNA decatenation reactions.

Each **DDC Spin-columns for DNA Decatenation Assays** (Catalog number DDC100) includes 100 DDC spin columns for 100 assays of DNA decatenation reactions. The DDC spin columns separate decatenated DNA from the concatenated DNA by a quick spin process.



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Protocol for spin column-based DNA decatenation assay

1. Reaction and sample preparation:

- (1) The total volume of each reaction mixture is 50 μ l. Step 1: mix 32.5 μ l of H₂O, 5 μ l of 10 x assay buffer, 5 μ l of 10 x concatenated DNA and 5 μ l of 2 mM ATP. Step 2, add 2.5 μ l of 20 x topoisomerase (1 U/ μ l) which is freshly diluted from the 200 x stock (10 U/ μ l) solution 10 fold using the dilution buffer, Step 3, incubate the reaction mixture at 37°C for 60 min.

Note: The final concentrations are 10 mM Tris-HCl, pH 8, 50 mM NaCl, 0.1 mM EDTA, 50 mM KCl, 5 mM MgCl₂, 15 μ g/ml BSA, 3 μ g/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.

- (2) Add 5 μ l 0.5 M EDTA to stop the reaction.

2. Column preparation:

- (1) Spin the column at 13000 rpm using a bench top Eppendorf centrifuge for 30 seconds to set down the resin.
- (2) Remove the column cap and bottom tip. Cut off the cap of a 1.5-Eppendorf tube. Place the column into the tube. Spin the column at 13000 rpm for 2 min. Transfer the column into a fresh Eppendorf tube.

3. Assay

- (1) Load the 50 μ l of the reaction mixture onto the column. Spin the column at 13000 rpm for 2 min. Collect the eluted solution from the spin column. Transfer the solution into a standard black 96-well plate well.
- (2) Dilute the 20 x fluorescence dye with water to make the 1 x fluorescence dye. Mix 150 μ l of the 1x fluorescence dye with the column eluent.
- (3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

96-well DNA decatenation assays

DNA decatenation assays in a 96-well plate format are available for DNA decatenation enzymes including human topoisomerase II and bacterial topoisomerase IV.

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

96-Well Human Topo II DNA Decatenation Assay Kit Plus
Human DNA Topoisomerase I Assay Kit Plus-100
Human Topoisomerase I, 100,000 Units

Catalog No. HDD96KE
Catalog No. HRA100KE
Catalog No. HTOPI-100

For more information of DNA topoisomerase assays and other drug target assays, please visit www.profoldin.com or contact technical support by email to info@profoldin.com.