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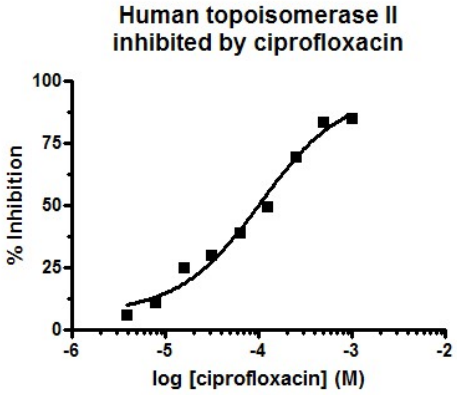
INSTRUCTIONS

ProFoldin
Spin-column DNA Topoisomerase Decatenation Assay Kits

DNA Topoisomerase IV Assay Kit	**Catalog No. DDC020K**
***E. coli* DNA Topoisomerase IV Assay Kit Plus**	**Catalog No. DDC020KE**
***E. coli* DNA Topoisomerase IV Assay Kit Plus-100**	**Catalog No. DDC100KE**
***S. aureus* DNA Topoisomerase IV Assay Kit Plus-100**	**Catalog No. DDC100KS**
***S. pneumoniae* DNA Topoisomerase IV Assay Kit Plus-100**	**Catalog No. DDC100KN**
***P. aeruginosa* DNA Topoisomerase IV Assay Kit Plus-100**	**Catalog No. DDC100KP**
Human Topoisomerase II DNA Decatenation Assay Kit Plus-100	**Catalog No. HDC100KE**
DDC Spin-columns for DNA Decatenation Assays	**Catalog No. DDC100**

Introduction

DNA topoisomerases such as bacterial DNA topoisomerase IV (the parC-parE complex) and human topoisomerase II convert the concatenated DNA into decatenated DNA during the DNA replication process in the cell. DNA decatenation activity by other topoisomerases including topoisomerase I and bacterial gyrases are also observed . The **Spin-column DNA Topoisomerase Decatenation Assays** are 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 **DNA Topoisomerase IV Assay Kit (Catalog No. DDC020K)** includes 150 µl of 10 x assay buffer (Buffer T4), 105 µl of 10 x concatenated DNA, 30 µl of 50 x ATP (10 mM),120 µl of 0.4 M EDTA, 160 µl of 20 x fluorescence dye and 20 spin columns for 20 DNA decatenation assays. The assay buffer is optimized for bacterial topoisomerase IV. Bacterial topoisomerase IV enzymes are provided separately.

The ***E. coli* DNA Topoisomerase IV Assay Kit Plus (Catalog No. DDC020KE)** includes all the components in **DNA Topoisomerase IV Assay Kit (Catalog No. DDC020K)** plus12 µl of 100 x *E.coli* topoisomerase IV (500 nM) for 20 *E. coli* topoisomerase IV DNA decatenation assays.

The ***E. coli* DNA Topoisomerase IV Assay Kit Plus-100 (Catalog No. DDC100KE)** includes the 600 µl of assay buffer (Buffer T4), 520 µl of 10 x concatenated DNA, 120 µl of 50 x ATP (10 mM), 550 µl of 0.4 M EDTA,

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780 µl of 20 x fluorescence dye, 100 spin columns and 55 µl of 100 x *E.coli* topoisomerase IV (500 nM) for 100 *E. coli* topoisomerase IV DNA decatenation assays.

The ***S. aureus*** **DNA Topoisomerase IV Assay Kit Plus-100 (Catalog No. DDC100KS)** includes all the reagents for 100 *S. aureus* topoisomerase IV DNA decatenation assays.

The ***S. pneumoniae*** **DNA Topoisomerase IV Assay Kit Plus-100 (Catalog No. DDC100KN)** includes all the reagents for 100 *S. pneumoniae* topoisomerase IV DNA decatenation assays.

The ***P. aeruginosa*** **DNA Topoisomerase IV Assay Kit Plus-100 (Catalog No. DDC100KP)** includes all the reagents for 100 *P. aeruginosa* topoisomerase IV DNA decatenation assays.

The **Human DNA Topoisomerase II Assay Kit Plus-100 (Catalog No. HDC100KE)** includes all the reagents for 100 Human topoisomerase II DNA decatenation assays.

The **DDC Spin-columns for DNA Decatenation Assays (Catalog No. DDC100)** includes 100 DDC spin columns for 100 DNA decatenation assays.

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Protocol for Bacterial Topoisomerase IV DNA Decatenation Assays

1. Reaction and sample preparation:

(1) The total volume of each reaction mixture is 50 µl including: 38.5 µl of H_2O, 5 µl of 10 x buffer, 5 µl of 10 x concatenated DNA, 0.5 µl of 10 x enzyme, 1 µl of 10 mM ATP. Incubate the reaction mixture at 37°C for 60 min. Add 5 µl 0.4 M EDTA to stop the reaction.

Note: The final concentrations are 20 mM Tris-HCl, pH 8, 35 mM NH_4OAc, 4.6 % glycerol, 1 mM DTT, 0.005% Brij35, 8 mM $MgCl_2$, 2 µg/ml concatenated DNA, 0.2 mM ATP and 5 nM topoisomerase IV. A negative control reaction can be the reaction mixture without addition of ATP.

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 µl of the reaction mixture onto the column. Spin the column at 13000 rpm for 2 min. Collect the solution eluted from the column.
(2) Dilute the 20 x fluorescence dye with water to make the 1 x fluorescence dye. Mix 150 µl of the 1x fluorescence dye with the solution eluted from the column.
(3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Reference

Narayanan S. et al. A cell cycle-controlled redox switch regulates the topoisomerase IV activity. Genes Dev. 29(11):1175-87 (2015).

Related products

DNA decatenation assays in 96-well plate format are available for high throughput screening of topoisomerase inhibitors. For more information of high throughput DNA topoisomerase assays and other drug target assays, please visit our website at http://www.profoldin.com.