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INSTRUCTIONS

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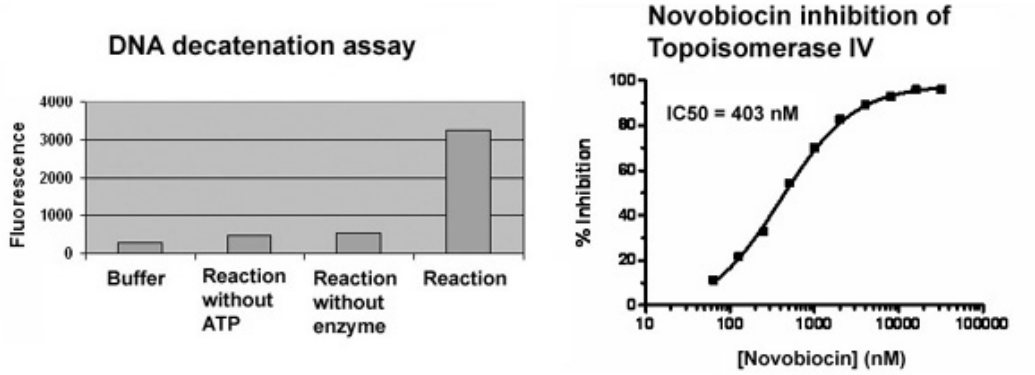
E. coli DNA topoisomerase IV (parC-parE complex)

***E. coli* DNA topoisomerase IV – for 100 assays** **Catalog No. TOP4-100EC**

Protein construct:	Wild-type *E. coli* topoisomerase IV composed of the parC and parE subunits forming a dimmer of $(parC)_2(parE_2)$.
MW:	308 kDa
Enzyme concentration:	5 µM
Enzyme activity assay:	The DNA decatenation activity is measured by using spin-columns (Catalog number: DDC100) or 96-well plates (Catalog number: TDD96K).
Storage temperature:	-20 or -80°C. Do not freeze-and-thaw repeatedly.
Enzyme dilution:	Use the 1 x assay to dilute the enzyme just before the assay.



The ***E. coli* DNA Topoisomerase IV – for 100 assays** (Catalog No. TOP4-100EC) 50 µl of 5 µM *E. coli* DNA topoisomerase IV (1000 x). It is for 100 assays.

DNA decatenation assay using spin-columns (Catalog No: DDC100)

1. Assay reaction and sample preparation:

The total volume of each reaction mixture is 50 µl including 30 µl of H_2O, 5 µl of 10 x Buffer T4, 5 µl of 10 x concatenated DNA, 5 µl of 10 x enzyme, 5 µl of 10 mM ATP. Incubate the reaction mixture at room temperature for 60 min. Stop the reaction with 5 µl of 0.5 M EDTA.

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 $MgCl_2$, 3 µg/ml concatenated DNA, 1 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.

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(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 loading sample onto the column. Spin the column at 13000 rpm for 2 min. Collect the eluted solution 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.

DNA decatenation assay using 96-well plates (Catalog No: TDD96K)

The following equipment is needed for the 96-well Topoisomerase DNA Decatenation Assay:

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.
A fluorescence reader:	A plate fluorescence reader with excitation at 485 nm and emission at 535 nm.

1. Assay reaction and sample preparation:

The total volume of each reaction mixture is 50 µl including 30 µl of H_2O, 5 µl of 10 x Buffer T4, 5 µl of 10 x concatenated DNA, 5 µl of 10 x enzyme, 5 µl of 10 mM ATP. Incubate the reaction mixture at room temperature for 60 min. Stop the reaction with 5 µl of 0.5 M EDTA.

2. Plate preparation:

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.

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

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

Related products:

DDC100	DDC Spin-columns for DNA decatenation assays
TDD96K	96-Well Topoisomerase DNA Decatenation Assay Kit

For more information of DNA topoisomerase assays and assays for more drug targets and enzymes, please visit www.profoldin.com or send emails to info@profoldin.com.