



## ProFoldin

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## INSTRUCTIONS

### ProFoldin

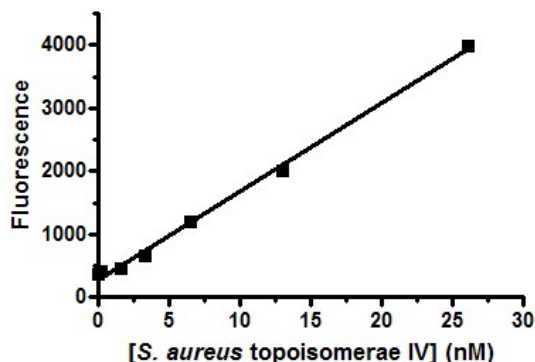
### *S. aureus* DNA topoisomerase IV (parC-parE complex)

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#### *S. aureus* DNA topoisomerase IV – for 100 assays    Catalog No. TOP4-100SA

Protein construct:	Wild-type <i>S. aureus</i> topoisomerase IV composed of the parC and parE subunits forming a dimer of (parC) <sub>2</sub> (parE) <sub>2</sub> .
MW:	330 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. Do not store diluted enzyme solution

***S. aureus* topoisomerase IV  
DNA decatenation assay**



The *S. aureus* DNA Topoisomerase IV – for 100 assays (Catalog No. TOP4-100SA) includes 50 μl of 5 μM *S. aureus* DNA topoisomerase IV (100 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<sub>2</sub>O, 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<sub>4</sub>OAc, 4.6 % glycerol, 1 mM DTT, 0.005% Brij35, 8 mM MgCl<sub>2</sub>, 3 μg/ml concatenated DNA, 1 mM ATP and 50 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l including 30  $\mu$ l of H<sub>2</sub>O, 5  $\mu$ l of 10 x Buffer T4, 5  $\mu$ l of 10 x concatenated DNA, 5  $\mu$ l of 10 x enzyme, 5  $\mu$ l of 10 mM ATP. Incubate the reaction mixture at room temperature for 60 min. Stop the reaction with 5  $\mu$ l 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  $\mu$ l of the sample onto the filter plate. Apply the vacuum (80 kpa or 600 mmHg) until the solution goes through the filter. Add 150  $\mu$ l of the Rinse Buffer and let the buffer completely go through the filter. Stop the vacuum and take out the receiver plate. Add 50  $\mu$ l 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](http://www.profoldin.com) or send emails to [info@profoldin.com](mailto:info@profoldin.com).