ProFoldin **Protein Folding Services**
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INSTRUCTIONS

ProFoldin
96-Well Topoisomerase DNA Decatenation Assay Kits

96-Well Topoisomerase DNA Decatenation Assay Kit	**Catalog No. TDD96K**
96-Well Human Topo II DNA Decatenation Assay Kit Plus	**Catalog No. HDD96KE**
96-Well *E. coli* Topo IV DNA Decatenation Assay Kit Plus	**Catalog No. EDD96KE**
96-Well *S. aureus* Topo IV DNA Decatenation Assay Kit Plus	**Catalog No. SDD96KE**
96-Well *S. pneumoniae* Topo IV DNA Decatenation Assay Kit Plus	**Catalog No. NDD96KE**
96-Well *P. aeruginosa* Topo IV DNA Decatenation Assay Kit Plus	**Catalog No. PDD96KE**
96-welll *E. coli* Topo I DNA Decatenation Assay Plus	**Catalog No. T1DD-96KE**
96-welll *E. coli* Gyrase DNA Decatenation Assay Kit Plus	**Catalog No. T2DD-96KE**
96-welll *S. aureus* Gyrase DNA Decatenation Assay Kit Plus	**Catalog No. T2DD-96KS**

Introduction

DNA decatenation is the major functions of topoisomerase II in human and topoisomerase IV (the parC-parE complex) in bacteria. Topoisomerase I and topoisomerase II in bacteria (gyrase) also have DNA decatenation activity. The DNA decatenation reaction converts the concatenated DNA into decatenated DNA. The **96-Well Topoisomerase DNA Decatenation Assays** are 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 96 well plate and quantified by fluorescence at 535 nm (excitation at 485 nm).

Each **96-Well Topoisomerase DNA Decatenation Assay Kit (Catalog No. TDD96K)** includes the assay buffer, concatenated DNA, ATP, stop solution, fluorescence dye, rinse buffer and a TDD filter plate for 96 assays of DNA decatenation reactions. The assay buffer is optimized for bacterial topoisomerase IV. DNA decatenation enzyme is not included.

Each **96-Well Human Topo II DNA Decatenation Assay Kit Plus (Catalog No. HDD96KE)** includes all reagents needed for 96 assays of human topo II DNA decatenation reactions.

Each **96-Well *E. coli* Topo IV DNA Decatenation Assay Kit Plus (Catalog No. EDD96KE)** includes all reagents needed for 96 assays of *E. coli* topo IV DNA decatenation reactions.

Each **96-Well *S. aureus* Topo IV DNA Decatenation Assay Kit Plus (Catalog No. SDD96KE)** includes all reagents needed for 96 assays of *S. aureus* topo IV DNA decatenation reactions.

Each **96-Well *S. pneumoniae* Topo IV DNA Decatenation Assay Kit Plus (Catalog No. NDD96KE)** includes all reagents needed for 96 assays of *S. pneumoniae* IV DNA decatenation reactions.

Each **96-Well *P. aeruginosa* Topo IV DNA Decatenation Assay Kit Plus (Catalog No. PDD96KE)** includes all reagents needed for 96 assays of *P. aeruginosa* topo IV DNA decatenation reactions.

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Each **96-welll *E. coli* Topo I DNA Decatenation Assay Plus (Catalog No. T1DD-96KE)** includes all reagents needed for 96 assays of *E. coli* topo I DNA decatenation reactions.

Each **96-welll *E. coli* Gyrase DNA Decatenation Assay Kit Plus (Catalog No. T2DD-96KE)** includes all reagents needed for 96 assays of *E .coli* gyrase DNA decatenation reactions.

Each **96-welll *S. aureus* Gyrase DNA Decatenation Assay Kit Plus (Catalog No. T2DD-96KS)** includes all reagents needed for 96 assays of *S, aureus* gyrase 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.
A receiver 96-well plate:	A standard black 96-well plate, well capacity 350 µl.
A fluorescence reader:	A plate fluorescence reader with excitation at 485 nm and emission at 535 nm.

Storage: -20°C except the TDD filter plate.

Assay Protocol

1. Reagent preparation and filtration unit

Dilute the 10 mM ATP with water 5-fold to make 2 mM ATP.
Dilute the 10 x fluorescence dye 10-fold to make 1x fluorescence dye.
Assembly the filtration unit by connecting the filtration device to a vacuum line, placing a standard black 96-well plate in the filtration device as a receiver of the filtration and the TDD filter plate on the top of the device.

2. Reaction and sample preparation:

The total volume of each reaction mixture is 50 µl including: 1 µl of inhibitor, 34.5 µl of H_2O, 5 µl of 10 x assay buffer, 5 µl of 10 x concatenated DNA, 5 µl of 2 mM ATP (for topoisomerase II or topoisomerase IV assays only), 0.5 µl of 100 x topoisomerase. Incubate the reaction mixture at room temperature for 30 to 60 min. Add 5 µl of Stop Solution to stop the reaction.

Note: The final concentrations for human topo II assay are 10 mM Tris-HCl, pH 8, 50 mM NaCl, 0.1 mM EDTA, 50 mM KCl, 5 mM $MgCl_2$, 0.015 % BSA, 2 µg/ml concatenated DNA, 0.2 mM ATP and 10 U/ml human topo II alpha.

The final concentrations for bacterial topoisomerase DNA decatenation assays 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 to 25 nM bacterial topoisomerases. ATP is not needed for topo I. A negative control reaction can be the reaction mixture without enzyme. For the topo IV and gyrase assays, the negative control can also be the reaction without ATP.

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.