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INSTRUCTIONS

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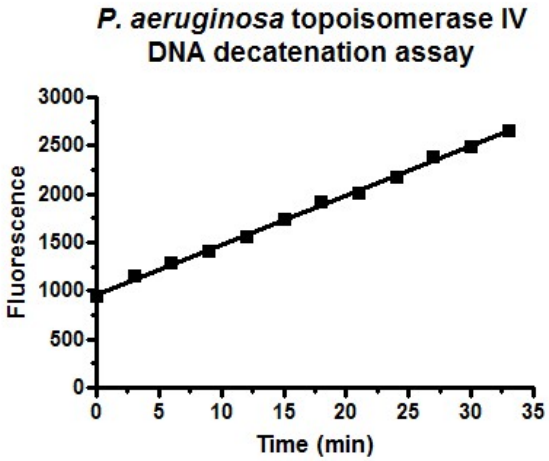
96-Well *P. aeruginosa* Topoisomerase IV DNA Decatenation Assay Kits

96-Well *P. aeruginosa* Topo IV DNA Decatenation Assay Kit Plus **Catalog No. PDD96KE**
96-Well Topoisomerase DNA Decatenation Assay Kit **Catalog No. TDD96K**

Introduction

DNA decatenation is an essential process during DNA replication in the cells. The DNA decatenation reaction was carried out by topoisomerase IV (the parC-parE complex) in bacteria. The DNA decatenation reaction converts the concatenated DNA into decatenated DNA. The **96-Well Topoisomerase DNA Decatenation Assay** is 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 black 96 well plate and quantified by fluorescence at 535 nm (excitation at 485 nm).



Each **96-Well *P. aeruginosa* Topoisomerase IV DNA Decatenation Assay Kit Plus (Catalog No. PDD96KE)** includes 600 µl of 10 x assay buffer, 500 µl of 10 x concatenated DNA, 110 µl of 10 mM ATP, 8 µl of 1000 x *P. aeruginosa* topo IV, 600 µl of 0.4 M EDTA, 260 µl of 20 x fluorescence dye, 2 ml of 10 x rinse buffer, one V-bottom plate, a TDD filter plate and one black 96-well plates for 96 assays of DNA decatenation reactions.

Each **96-Well Topoisomerase DNA Decatenation Assay Kit (Catalog No. TDD96K)** includes 600 µl of 10 x assay buffer, 500 µl of 10 x concatenated DNA, 110 µl of 10 mM ATP, 600 µl of 0.4 M EDTA, 260 µl of 20 x fluorescence dye, 2 ml of 10 x rinse buffer, one V-bottom plate, a TDD filter plate and one black 96-well plates for 96 assays of DNA decatenation reactions. The assay buffer is optimized for bacterial topoisomerase IV. DNA decatenation enzyme is not included. This kit can be used for DNA decatenation assays of any bacterial topoisomerase IV or the parC-parE complexes.

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

Assay Protocol

1. Reagent preparation and filtration unit

Dilute the 10 mM ATP with water 5-fold to make 2 mM ATP.
Dilute the 20 x fluorescence dye 20-fold to make 1x fluorescence dye.
Dilute the 10 x Rinse buffer 10-fold with water to make 1 x Rinse buffer.
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.

2. Reaction and sample preparation:

The reactions are carried out in a V-bottom plate. 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, 0.5 µl of 100 x topoisomerase. Incubate the reaction mixture at 37°C for 60 min. Add 5 µl of Stop Solution to stop the reaction.

Note: 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 $MgCl_2$, 3 µg/ml concatenated DNA, 0.2 mM ATP and 5 nM *P. aeruginosa* topoisomerase IV. A negative control reaction can be the reaction mixture without enzyme or 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.

Related products

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 *E. coli* Topo I DNA Decatenation Assay Plus	Catalog No. T1DD-96KE

For more information of DNA topoisomerase assays, please visit the website of The Topo World: http://www.profoldin.com/topoisomerase_assays_1.html.

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

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