



**ProFoldin** Protein Folding Services  
290 Turnpike Road, Suite 6, Number 321  
Westborough, MA 01581-2843  
FAX: (508) 845-9258  
[www.profoldin.com](http://www.profoldin.com)  
[info@profoldin.com](mailto:info@profoldin.com)

## INSTRUCTIONS

# ProFoldin DNA Topoisomerase IV Assay Kit

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**Catalog Number**            **DDC0020K**

### Introduction

DNA topoisomerase IV (the parC-parE complex) converts the concatenated DNA into decatenated DNA. This reaction is called DNA decatenation reaction. The DNA Topoisomerase IV Assay Kit is based 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.

Each kit (Catalog number DDC020K) includes the assay buffer, concatenated DNA, loading solution and spin columns for 20 assays of DNA decatenation reactions. The assay buffer is optimized for bacterial topoisomerase IV.

### Assay Protocol

#### 1. Reaction and sample preparation:

(1) The total volume of each reaction mixture is 25  $\mu$ l including: 15  $\mu$ l of H<sub>2</sub>O, 2.5  $\mu$ l of 10 x buffer, 2.5  $\mu$ l of 10 x concatenated DNA, 2.5  $\mu$ l of 10 x enzyme, 2.5  $\mu$ l of 10 mM ATP. Incubate the reaction mixture at room temperature for 30 min.

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>, 2  $\mu$ g/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) Mix 25  $\mu$ l of loading solution with the 25  $\mu$ l the reaction solution to makes 50  $\mu$ l of the loading sample for each reaction.

#### 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  $\mu$ l of the loading sample onto the column. Spin the column at 13000 rpm for 2 min. Collect the column eluent.

(2) Dilute the commercially available 10,000 x SYBR Green II reagent with water 2000-fold to make the 1 x fluorescence dye. Mix 150  $\mu$ l of the 1x fluorescence dye with the column eluent.

(3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.