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## INSTRUCTIONS

# ProFoldin DNA Topoisomerase I Assay Kit

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**Catalog Number**                      **DRA020K**

### Introduction

DNA topoisomerases such as bacterial topoisomerase I convert supercoiled circular DNA into relaxed DNA. This reaction is called DNA relaxation reaction. The DNA Topoisomerase I Assay Kit is based on the principle that the supercoiled DNA and relaxed DNA yield different fluorescent intensity when interact with fluorescence dye H19 (a fluorescence dye for DNA relaxation / supercoiling assay). The relaxed DNA suppresses the fluorescent intensity much more than the supercoiled DNA. Therefore, when the supercoiled DNA is converted into its relaxed form, the fluorescent signal decreases. The change of fluorescence intensity is used to measure the relaxation reaction.

Each kit (Catalog number DRA020K) includes the reaction buffer (Buffer T1), supercoiled plasmid DNA, reaction terminator and fluorescence dye H19 for 20 assays of DNA relaxation reactions in a 96-well plate format or 40 assays in a 384-well assay format. The following protocol is for the assays in 96-well plates. Please adjust the reagent volumes accordingly for assays in 384-well plates. The reaction buffer is optimized for bacterial topoisomerase I. The 1 x reaction buffer can be used for dilution of the enzyme solution.

### Assay Protocol

#### 1. Reaction and sample preparation:

The total volume of each reaction mixture is 40  $\mu$ l including: 28  $\mu$ l of H<sub>2</sub>O, 4  $\mu$ l of 10 x Buffer T1, 4  $\mu$ l of 10 x supercoiled DNA, 4  $\mu$ l of 10 x enzyme. Incubate the reaction mixture at room temperature for 60 min.

Note: The final concentrations are 16 mM Tris-HCl, pH 8, 6 mM MgCl<sub>2</sub>, 25  $\mu$ g/ml supercoiled plasmid DNA and 40 nM topoisomerase I.

#### 2. Assay

- (1) Dilute the 100 x H19 dye with 10 mM Tris-HCl, 10 mM NaCl, pH 7.0 to make 1 x H19 dye.
- (2) Mix 50  $\mu$ l of the diluted H19 dye with each reaction solution. Incubate the mixture at room temperature for 5 min.
- (3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Note: Fluorescence signals are sensitive to temperature changes. Please keep the temperature consistent during the measurement.