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

INSTRUCTIONS

ProFoldin
DNA Topoisomerase I Assay Kits

DNA Topoisomerase I Assay Kit **Catalog No. DRA020K**
DNA Topoisomerase I Assay Kit Plus **Catalog No. DRA020KE**
DNA Topoisomerase I Assay Reagent Set for HTS **Catalog No. DRA1000**

Introduction

DNA topoisomerases such as bacterial topoisomerase I convert supercoiled circular DNA into relaxed DNA (DNA relaxation reaction). The **DNA Topoisomerase I Assay Kit** is based on the principle that the relaxed DNA suppresses the fluorescent intensity much more than the supercoiled DNA when the DNA interacts with fluorescence dye H19 (a fluorescence dye for DNA relaxation / supercoiling assay) in the presence of magnesium. When the supercoiled DNA is converted into its relaxed form, the fluorescent signal decreases.

The **DNA Topoisomerase I Assay Kit** (Catalog number DRA020K) includes the reaction buffer (Buffer T1), supercoiled plasmid DNA, 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 kit does not include *E. coli* topoisomerase I that can be purchased separately (Catalog number TOP1-100EC).

The **DNA Topoisomerase I Assay Kit Plus** (Catalog No. DRA020KE) includes the kit reagents of DRA020K plus *E. coli* topoisomerase I.

The **DNA Topoisomerase I Assay Reagent Set for HTS** (Catalog No. DRA1000) includes the reaction buffer (Buffer T1) and fluorescence dye H19 for 1000 assays of DNA relaxation reactions in a 96-well or 384-well assay format. DNA or topoisomerase I is not included. Commercially available supercoiled pBR322 or pUC19 plasmid DNA can be used for the assay.

Related Products:

TOP1-100EC *E. coli* topoisomerase I

Assay Protocol

1. Reaction and sample preparation:

The total volume of each reaction mixture is 40 µl including: 28 µl of H_2O, 4 µl of 10 x Buffer T1, 4 µl of 10 x supercoiled DNA, 4 µl of 10 x Topoisomerase I. Incubate the reaction mixture at room temperature for 60 min.

Note: The final concentrations are 16 mM Tris-HCl, pH 8, 6 mM $MgCl_2$, 25 µg/ml supercoiled plasmid DNA and 40 nM topoisomerase I. EDTA should be avoided in the reaction.

2. Assay

(1) Freshly prepare the 1 x H19 dye by dilution of the 100 x H19 dye with 10 mM Tris-HCl, 10 mM NaCl, pH 7.0.
(2) Mix 50 µl of the diluted H19 dye with each reaction solution. Incubate the mixture for 5 min.
(3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.