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INSTRUCTIONS

ProFoldin
Human DNA Topoisomerase I Assay Kits

Human DNA Topoisomerase I Assay Kit **Catalog No. HRA020K**
Human Topoisomerase I DNA Relaxation Assay HTS Kit **Catalog No. HRA1000**

Introduction

Human DNA topoisomerase I converts supercoiled circular DNA into relaxed DNA (DNA relaxation). The **Human DNA Topoisomerase I Assay Kit** is based on the principle that the supercoiled DNA and relaxed DNA yield different fluorescent intensity when interact with the fluorescence dye for DNA relaxation assay (fluorescence dye H19). 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.

The **Human DNA Topoisomerase I Assay Kit** (Catalog number HRA020K) includes the reaction buffer (Buffer HT), 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 Human DNA topoisomerase I that can be purchased from Sigma, catalog number T9069.

The **Human DNA Topoisomerase I Assay Reagent Set for HTS** (Catalog number HRA1000) includes the reaction buffer (Buffer HT) and fluorescence dye H19 for 1000 assays of DNA relaxation reactions in a 96-well plate format or 2000 assays in a 384-well assay format.

Related products:

HDC020K	Human Topoisomerase II DNA Decatenation Assay Kit
DRA020K	DNA Topoisomerase I Assay Kit
DSA020K	DNA Topoisomerase II (Gyrase) Assay Kit
DDC020K	DNA Topoisomerase IV Assay Kit

Assay Protocol

1. **Reaction and sample preparation:**

   The total volume of each reaction mixture is 40 µl including: 28 µl of H2O, 4 µl of 10 x Buffer HT, 4 µl of 10 x supercoiled DNA, 4 µl of 50 U/ml human topoisomerase I. Incubate the reaction mixture at room temperature for 60 min.

   Note: The final concentrations are 10 mM Tris-HCl, pH 8, 50 mM NaCl, 0.1 mM EDTA, 50 mM KCl, 5 mM MgCl2, 0.015 % BSA, 25 µg/ml supercoiled plasmid DNA and 5 U/ml topoisomerase I.

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2. Assay

(1) Freshly 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 μl 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.