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INSTRUCTIONS

ProFoldin Human Topoisomerase II DNA Decatenation Assay Kit

Catalog Number **HDC020K**

Introduction

Human DNA topoisomerase II alpha converts the concatenated DNA into decatenated DNA. This reaction is called DNA decatenation reaction. The **Human Topoisomerase II DNA Decatenation 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 HDC020K) includes the assay buffer, concatenated DNA and spin columns for 20 assays of DNA decatenation reactions. The assay buffer is optimized for human topoisomerase II alpha.

Assay Protocol

1. Reaction and sample preparation:

(1) The total volume of each reaction mixture is 25 μ l including: 15 μ l of H₂O, 2.5 μ l of 10 x buffer, 2.5 μ l of 10 x concatenated DNA, 2.5 μ l of 10 x enzyme, 2.5 μ l of 10 mM ATP. Incubate the reaction mixture at room temperature for 30 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, 1 mM ATP and 10 U/ml human topoisomerase II alpha. A negative control reaction can be the reaction mixture without addition of ATP.

(2) Mix 25 μ l of loading solution with the 25 μ l the reaction solution to makes 50 μ 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 μ 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 μ 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.