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INSTRUCTIONS

ProFoldin DNA Topoisomerase II (Gyrase) Assay Kit

Catalog Number **DSA020K**

Introduction

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

Each kit (Catalog number DSA020K) includes the reaction buffer (T2 Buffer), relaxed plasmid DNA, reaction terminator and fluorescence dye H19 for 20 assays of DNA supercoiling 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 II (gyrase). 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 μ l including: 24 μ l of H₂O, 4 μ l of 10 x buffer, 4 μ l of 10 x relaxed DNA, 4 μ l of 10 x enzyme, 4 μ l of 10 mM ATP. Incubate the reaction mixture at room temperature for 60 min. At the end of the reaction, add 200 μ l of H₂O into each reaction mixture.

Note: The final concentrations are 20 mM Tris-HCl, pH 8, 35 mM NH₄OAc, 4.6 % glycerol, 1 mM DTT, 0.005% Brij35, 8 mM MgCl₂, 25 μ g/ml relaxed plasmid DNA, 1 mM ATP and 20 nM topoisomerase II. A negative control reaction can be the reaction mixture without addition of ATP.

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 μ l of the diluted H19 dye with each reaction solution. Incubate the mixture at room temperature for 15 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.