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INSTRUCTIONS

ProFoldin
DNA Gyrase DNA Supercoiling Assay Kits

DNA Topoisomerase II (Gyrase) Assay Kit	**Catalog No. DSA020K**
DNA Topoisomerase II (Gyrase) Assay Kit Plus	**Catalog No. DSA020KE**
***S. aureus* Gyrase DNA Supercoiling Assay Plus**	**Catalog No. DSA020KSE**
DNA Topoisomerase II Assay Reagent Set for HTS	**Catalog No. DSA1000**

Introduction

DNA topoisomerases such as bacterial topoisomerase II (gyrase) convert relaxed circular DNA into supercoiled DNA (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 in the presence of magnesium. 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 of gyrases and high throughput screen of gyrase inhibitors.

The **DNA Topoisomerase II (Gyrase) Assay Kit** (Catalog number DSA020K) includes the reaction buffer (T2 Buffer), relaxed plasmid DNA, ATP 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 reaction buffer is optimized for gram-negative bacterial topoisomerase II (gyrase). For *S. aureus* gyrase, 400 mM potassium glutamate should be included in the reaction buffer. The kit does not include the DNA topoisomerase that can be purchased separately (please see the related products).

The **DNA Topoisomerase II (Gyrase) Assay Kit Plus** (Catalog No. DSA020KE) includes the kit reagents of DSA020K plus *E. coli* topoisomerase II (gyrase).

The ***S. aureus* Gyrase DNA Supercoiling Assay Plus** (Catalog No. DSA020KSE) includes the kit reagents of DSA020K plus *S. aureus* topoisomerase II (gyrase).

The **DNA Topoisomerase II Assay Reagent Set for HTS** (Catalog No. DSA1000) includes the reaction buffer (T2 Buffer) and fluorescence dye H19 for 1000 assays of DNA supercoiling reactions in a 96-well plate format or 2000 assays in a 384-well assay format.

Assay Protocol

1. **Reaction and sample preparation:**

   The total volume of each reaction mixture is 40 µl including: 24 µl of H_2O, 4 µl of 10 x buffer, 4 µl of 10 x relaxed DNA, 4 µl of 10 x enzyme, 4 µl of 10 mM ATP. Incubate the reaction mixture at

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room temperature for 60 min. At the end of the reaction, add 200 µl of H_2O into each reaction mixture.

Note: The final concentrations are 20 mM Tris-HCl, pH 8, 35 mM NH_4OAc, 4.6 % glycerol, 1 mM DTT, 0.005% Brij35, 8 mM $MgCl_2$, 25 µg/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. Magnesium is essential for the reaction and assay. EDTA should be avoided.

For *S. aureus* gyrase, a final concentration of 400 mM potassium glutamate should be added into the reaction buffer. A concentration of 50 to 100 nM *S. aureus* gyrase is used.

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 freshly prepared 1 x H19 dye with each reaction solution (240 µl). Incubate the mixture at room temperature for 15 min.
(3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Related Products:

96-welll *E. coli* Gyrase DNA Decatenation Assay Kit Plus	Catalog No. T2DD-96KE
96-welll *S. aureus* Gyrase DNA Decatenation Assay Kit Plus	Catalog No. T2DD-96KS
E. coli gyrase ATPase assay Kit Plus	Catalog No. T2A-100KE
S. aureus gyrase ATPase assay Kit Plus	Catalog No. T2A-100KS
96-well *E. coli* Gyrase DNA Cleavage Assay Kit Plus	Catalog No. T2C96KE