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INSTRUCTIONS

ProFoldin
Relaxed and Supercoiled Plasmid DNA Substrates

Relaxed plasmid DNA - 0.050 mg	**Catalog No. RDNA-050UG**
Relaxed Plasmid DNA - 1 mg	**Catalog No. RDNA-1MG**
Supercoiled plasmid DNA - 0.050 mg	**Catalog No. SDNA-050UG**
Supercoiled Plasmid DNA -1 mg	**Catalog No. SDNA-1MG**

DNA size: 5 kbp
DNA concentration: 1 mg /ml
Buffer: 10 mM Tris-HCl, 2 mM EDTA, pH 7.5

Introduction

The relaxed plasmid DNA is a substrate of DNA supercoiling enzymes such as bacterial gyrases. The supercoiled DNA is a substrate of DNA relaxation enzymes such as DNA topoisomerase I and topoisomerase IV. The DNA supercoiling and relaxation reactions can be monitored by fluorescence dye H19 based on the principle that the supercoiled DNA and relaxed DNA yield different fluorescent intensity when the DNA interacts 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 by a gyrase, the fluorescent signal increases. When the supercoiled DNA is converted into its relaxed form by topoisomerase I, the fluorescent signal decreases. The change of fluorescence intensity is used for characterization of topoisomerases and high throughput screen of gyrase and topoisomerase I inhibitors.

DNA supercoiling 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 (250 µg/ml relaxed plasmid DNA), 4 µl of 10 x enzyme, 4 µl of 10 mM ATP. Incubate the reaction mixture at room temperature for 60 min.

The assay reaction and detection require a free magnesium concentration between 2 mM to 10 mM. 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 H19 dye by dilution of 1 µl the 1500 x H19 dye stock with 1.5 ml of the 1 x H19 dilution buffer (1500 x dilution).

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(2) Mix 250 µl of the freshly prepared H19 dye with each reaction solution (40 µl). Incubate the mixture at room temperature for 5 min.
(3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Human Topoisomerase I DNA Relaxation 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 HT1, 4 µl of 10 x supercoiled DNA (250 µg/ml), 4 µl of 10 x human topoisomerase I (1000 U/ml). Incubate the reaction mixture at 37°C for 30 to 60 min.

Note: The final concentrations are 25 mM Tris-HCl, pH 7.4, 58 mM KCl, 0.25 mM DTT, 5 mM $MgCl_2$, 0.25 mM EDTA, 15 µg/ml BSA, 25 µg/ml supercoiled plasmid DNA and 100 U/ml topoisomerase I. A negative control reaction can be the reaction mixture without the enzyme. If the assay temperature is lower than 37°C, a higher enzyme concentration will be needed.

3. Assay

(1) For each 10 assays, freshly dilute 2 µl of 1500 x H19 dye into 3 ml of 1x H19 dilution buffer. The 1x H19 dilution buffer is prepared by 10 fold dilution of the 10x H19 dilution buffer with water.
(2) Mix 250 µl of the freshly prepared 1 x H19 dye with each reaction solution (40 µl). Incubate the mixture at room temperature for 5 min.
(3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Related Products:

Human topoisomerase I, 40,000 Units	Catalog No. HTOPI-040
Human topoisomerase I, 100,000 Units	Catalog No. HTOPI-100
10 x Assay Buffer for Human DNA Topoisomerase I Assays	Catalog No. HT1BUF-100
H19 Dye for DNA Relaxation and Supercoiling Assays	Catalog No. DSA1000D
Human topoisomerase I DNA relaxation assay kit plus-100	Catalog No. HRA100KE
Human topoisomerase I DNA relaxation assay kit plus-1000	Catalog No. HRA1000KE
DNA Topoisomerase II (Gyrase) Assay Kit Plus-100	Catalog No. DSA100KE
S. aureus Gyrase DNA Supercoiling Assay Plus-100	Catalog No. DSA100KSE
E. coli gyrase ATPase assay Kit Plus	Catalog No. T2A-100KE
S. aureus gyrase ATPase assay Kit Plus	Catalog No. T2A-100KS
96-well Topoisomerase DNA cleavage assay kit	Catalog No. TDC96K
DNA Topoisomerase I Assay Kit Plus-100	Catalog No. DRA100KE

Publications

1. Asha M.K. et al, In vitro anti-Helicobacter pylori activity of a flavonoid rich extract of Glycyrrhiza glabra and its probable mechanisms of action, Journal of Ethnopharmacology, Vol.145(2), pp.581-586 (2013).
2. Mora-Pale M et al, Antimicrobial mechanism of resveratrol-trans-dihydrodimer produced from peroxidase-catalyzed oxidation of resveratrol. Biotechnol Bioeng.Vol 112, pp2417–2428 (2015).