



ProFoldin

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INSTRUCTIONS

ProFoldin

E. coli DNA Helicase ATPase Assay Kits

E. coli DNA Helicase ATPase assay Kit Plus-100

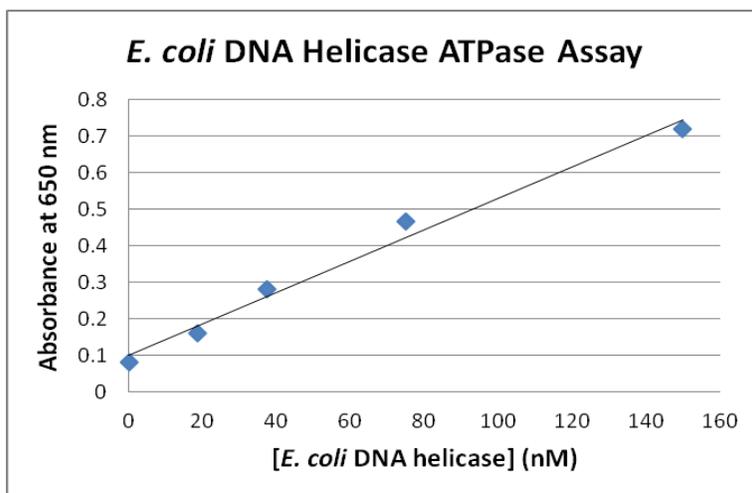
Catalog No. DNAB100KE

E. coli DNA Helicase ATPase assay Kit Plus-500

Catalog No. DNAB500KE

Introduction

DNA helicase (DnaB) hydrolyzes ATP as the source of molecular energy to carry out DNA unwinding required by the DNA replication process. Inhibition of the ATPase activity of DNA helicase blocks its DNA unwinding function. The DNA helicase ATPase assay can be used for high-throughput screen of DNA helicase inhibitors in drug discovery. The **DNA Helicase ATPase Assay Kit** is based on detection of the phosphate produced by the ATP hydrolysis reaction in the presence of DNA. The assay is in a 384-well plate format and the phosphate is detected using light absorbance at 650 nm.



The *E. coli* DNA Helicase ATPase assay Kit Plus-100 (Catalog No. DNAB100KE) includes 500 μ l of 10 x assay buffer, 35 μ l of 100 x DNA, 30 μ l of 100 x *E. coli* DNA helicase, 35 μ l of 100 x ATP and 5 ml of dye for 100 assays in a 384-well assay format.

The *E. coli* DNA Helicase ATPase assay Kit Plus-500 (Catalog No. DNAB500KE) includes 2 ml of 10 x assay buffer, 170 μ l of 100 x DNA, 150 μ l of 100 x *E. coli* DNA helicase, 170 μ l of 100 x ATP and 25 ml of dye for 500 assays in a 384-well assay format.

Publication

Nakano T., et al, Translocation and Stability of Replicative DNA Helicases upon Encountering DNA-Protein Cross-links, J. Biol. Chem. 288: 4649-4658 (2013).



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Assay Protocol

1. Reagent preparation:

For each 10 assay reactions,

- (1) Prepare 297 μ l of premix composed of 261 μ l of H₂O, 33 μ l of 10 x Buffer and 3.3 μ l of 100 x *E. coli* DNA helicase.
- (2) Prepare 33 μ l of 10 x Enzyme substrate by mixing 3.3 μ l of 100 x ATP and 3.3 μ l of 100 x DNA and 26.4 μ l of water.

2. Reaction:

Mix 27 μ l of the premix with 3 μ l of the 10 x Enzyme substrate in each well. Incubate the reaction mixture at 37°C for 60 min.

Note: The final concentrations for the ATPase assays of the helicases are 20 mM HEPES, pH 7.5, 20 mM potassium glutamate, 1 mM DTT, 0.005% Triton X-100, 10 mM MgCl₂, 250 nM DNA, 0.25 mM ATP and 200 nM DNA helicase. A negative control reaction can be the reaction mixture without addition of ATP or enzyme.

3. Detection:

Add 45 μ l of the Dye MPA3000 into the 30 μ l of the reaction mixture. Incubate for 5 min. Measure the light absorbance at 650 nm.

Assay optimization for enzyme inhibition

The assay can be optimized in terms of assay window, assay linearity and sensitivity to competitive inhibitors.

ProFoldin offers HTS assay development service. For more information, please visit our website at

<http://www.profoldin.com/services.html>.

Related products

<i>H. influenzae</i> DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KH
<i>S. pneumoniae</i> DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KN
<i>P. aeruginosa</i> DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KP
<i>S. aureus</i> DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KS
<i>E. coli</i> DNA Primase Assay Kit Plus	Catalog No. EGA100KE
<i>H. influenzae</i> DNA Primase Assay Kit Plus	Catalog No. HGA100KE
<i>S. pneumoniae</i> DNA Primase Assay Kit Plus	Catalog No. PGA100KE
<i>S. aureus</i> DNA Primase Assay Kit Plus	Catalog No. AGA100KE

For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.