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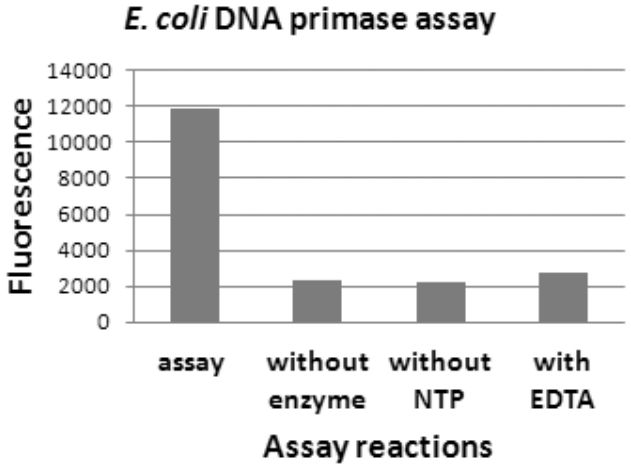
INSTRUCTIONS

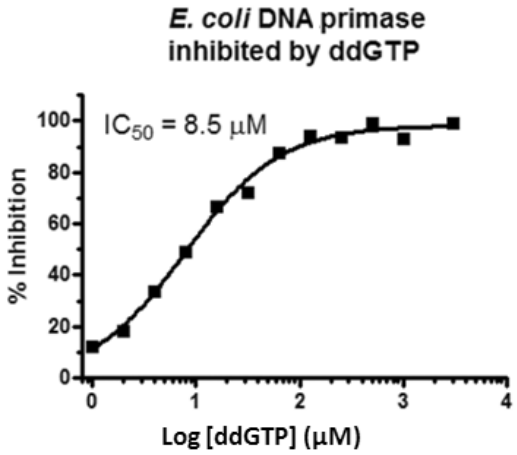
ProFoldin
E. coli DNA Primase Assay Kits

***E. coli* DNA Primase Assay Kit** **Catalog No. EGA100K**
***E. coli* DNA Primase Assay Kit Plus** **Catalog No. EGA100KE**
***E. coli* DNA Primase Assay Kit Plus-500** **Catalog No. EGA500KE**

Introduction

The bacterial DNA primase (DnaG) synthesizes RNA primers at the DNA replication fork where the DNA helicase (DnaB) unwinds the double strand DNA. The **Bacterial DNA Primase Assays** are based on measurement of the RNA primers synthesized by the DNA primase in the presence of the DNA temperate and NTPS. DNA helicases dramatically stimulate the activities of gram-negative bacterial primases (e.g. *E. coli* and *H. influenzae*) but not gram-positive bacterial primases (*S. aureus* and *S. pneumoniae*). The assays can be performed in a 96-well plate or 384-well plate format for high throughput screening of DNA primase inhibitors.





The ***E. coli* DNA Primase Assay Kit** (Catalog No. EGA100K) includes 600 µl of 10 x assay buffer, 45 µl of 100 x DNA template, 45 µl of 100 x NTP mix and 850 µl of 10 x fluorescence dye for 100 assays of *E. coli* DNA primase reactions in a 96-well plate format or 200 assays in 384-well assay format. The kit does not include the enzyme.

The ***E. coli* DNA Primase Assay Kit Plus** (Catalog No. EGA100KE) includes all the kit components in ***E. coli* DNA Primase Assay Kit** (Catalog No. EGA100K) plus 45 µl of 100 x *E. coli* primase-helicase complex. It is for 100 assays of *E.coli* DNA primase reactions in a 96-well plate format or 200 assays in 384-well assay format.

The ***E. coli* DNA Primase Assay Kit Plus-500** (Catalog No. EGA500KE) includes all the assay components including the enzyme for 500 assays in a 96-well plate format or 1000 assays in 384-well assay format.

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Assay Protocol

1. **Reagent preparation:**
   10 x DNA: dilute the 100 x DNA with water.
   10 x enzyme: Dilute the 100 x enzyme stock with the 1 x assay buffer to make the 10 x enzyme.
   10 x NTP mix: dilute the 100 x NTP 10-fold with water.
   1 x fluorescence dye: dilute the 10 x fluorescence dye 10-fold with water.

2. **Reaction**:
   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 DNA template, 4 µl of 10 x enzyme, 4 µl of 10 x NTP mix. Incubate the reaction mixture at 37°C for 60 min.

   Note: The assay solution is composed of 10 mM HEPES, pH 7.5, 5 mM magnesium acetate, 0.5 mM DTT, 0.003% Brij-35, 100 nM DNA, 0.5 mM NTPs, 100 nM enzyme.

3. **Detection**:
   Add 80 µl of the 1 x fluorescence dye into the 40 µl of the reaction mixture. Incubate for 5 min. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 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.

Reference

Lacriola CJ et al, Inhibition of DNA replication in Staphylococcus aureus by tegaserod. The Journal of Antibiotics. 70, 918–920 (2017).

Related Products:

H. influenzae DNA Primase Assay Kit Plus	Catalog No. HGA100KE
S. aureus DNA Primase Assay Kit Plus	Catalog No. AGA100KE
S. pneumoniae DNA Primase Assay Kit Plus	Catalog No. PGA100KE

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