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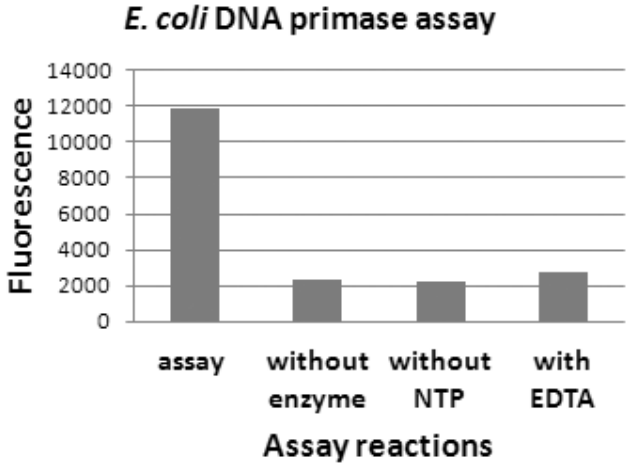
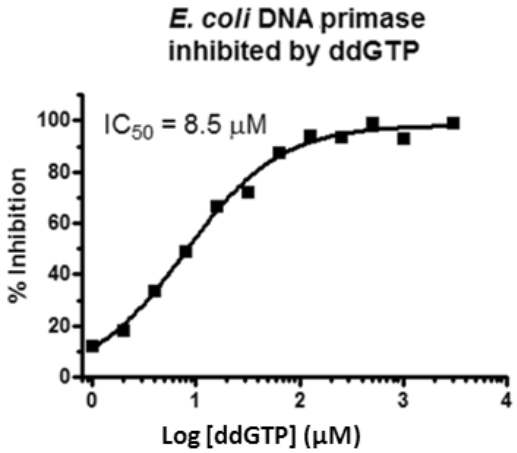
INSTRUCTIONS

ProFoldin
E. coli DNA Primase

E. coli DNA Primase – for 100 assays Catalog No. DNAG-100EC

Protein construct:	Wild-type *E. coli* DNA primase purified from a bacterial expression system.
MW:	65 kDa
Enzyme concentration:	10 µM
Enzyme activity assay:	The DNA primase activity is measured by using the *E. coli* DNA Primase Assay Kit (Catalog No. EGA100K).
Storage temperature:	-20 or -80°C. Do not freeze-and-thaw repeatedly.
Enzyme dilution:	Use the 1 x assay to dilute the enzyme just before the assay. Do not store diluted enzyme solution





The ***E. coli* DNA Primase – for 100 assays** (Catalog No. DNAG-100EC) includes 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.

Assay Protocol using the *E. coli* DNA Primase Assay Kit

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.

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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:

E. coli DNA Primase Assay Kit	Catalog No. EGA100K
E. coli DNA Primase Assay Kit Plus	Catalog No. EGA100KE
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
E. coli DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KE
H. influenzae DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KH
S. pneumoniae DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KN
P. aeruginosa DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KP
S. aureus DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KS

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