



ProFoldin

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INSTRUCTIONS

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H. influenzae DNA Primase Assay Kits

H. influenzae DNA Primase Assay Kit

Catalog No. HGA100K

H. influenzae DNA Primase Assay Kit Plus

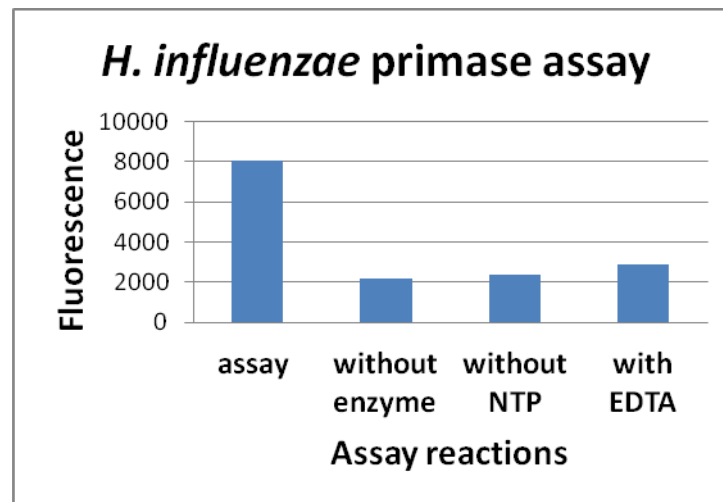
Catalog No. HGA100KE

H. influenzae DNA Primase Assay Kit Plus-500

Catalog No. HGA500KE

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 template 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 *H. influenzae* DNA Primase Assay Kit (Catalog No. HGA100K) includes 600 μ l of 10 x assay buffer, 45 μ l of 100 x DNA template, 45 μ l of 100 x NTP mix and 850 μ l of 10 x fluorescence dye for 100 assays of *H. influenzae* 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 *H. influenzae* DNA Primase Assay Kit Plus (Catalog No. HGA100KE) includes all the kit components in *H. influenzae* DNA Primase Assay Kit (Catalog No. HGA100K) plus 45 μ l of 100 x *H. influenzae* primase-helicase complex. It is for 100 assays of *H. influenzae* DNA primase reactions in a 96-well plate format or 200 assays in 384-well assay format.

The *H. influenzae* DNA Primase Assay Kit Plus-500 (Catalog No. HGA500KE) 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 μ l including 24 μ l of H₂O, 4 μ l of 10 x Buffer, 4 μ l of 10 x DNA template, 4 μ l of 10 x enzyme, 4 μ l of 10 x NTP mix. Incubate the reaction mixture at 37°C for 60 min.

Note: The assay solution is composed of 10 mM Tris-HCl, pH 7.5, 5 mM magnesium acetate, 12.5 mM ammonium chloride, 0.5 mM DTT, 2.5 mM calcium chloride, 0.003% Brij-35, 100 nM DNA, 0.5 mM NTPs, 100 nM enzyme.

3. Detection:

Add 80 μ l of the 1 x fluorescence dye into the 40 μ l 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 Plus

Catalog No. EGA100KE

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.