

**ProFoldin Protein Folding Services**

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## INSTRUCTIONS

# ProFoldin

## Bacterial DNA Primase Assay Kits

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<i>E. coli</i> DNA Primase Assay Kit	Catalog No. EGA100K
<i>E. coli</i> DNA Primase Assay Kit Plus	Catalog No. EGA100KE
<i>H. influenzae</i> DNA Primase Assay Kit	Catalog No. HGA100K
<i>H. influenzae</i> DNA Primase Assay Kit Plus	Catalog No. HGA100KE
<i>S. aureus</i> DNA Primase Assay Kit	Catalog No. AGA100K
<i>S. aureus</i> DNA Primase Assay Kit Plus	Catalog No. AGA100KE
<i>S. pneumoniae</i> DNA Primase Assay Kit	Catalog No. PGA100K
<i>S. pneumoniae</i> DNA Primase Assay Kit Plus	Catalog No. PGA100KE

### Introduction

The bacterial DNA primase synthesizes RNA primers at the DNA replication fork where the DNA helicase (DnaB) unwinds the double strand DNA. The **Bacterial DNA Primase Assay Kits** 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 assay 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 the assay buffer, DNA template, NTP mix and fluorescence dye for 100 assays of 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 the assay buffer, DNA template, NTP mix and fluorescence dye for 100 assays of DNA primase reactions in a 96-well plate format or 200 assays in 384-well assay format. The kit includes *E. coli* primase-helicase complex.

The *H. influenzae* DNA Primase Assay Kit (Catalog No. HGA100K) includes the assay buffer, DNA template, NTP mix and fluorescence dye for 100 assays of 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 the assay buffer, DNA template, NTP mix and fluorescence dye for 100 assays of DNA primase reactions in a 96-well plate format or 200 assays in 384-well assay format. The kit includes *H. influenzae* primase-helicase complex.

The *S. aureus* DNA Primase Assay Kit (Catalog No. AGA100K) includes the assay buffer, DNA template, NTP mix and fluorescence dye for 100 assays of DNA primase reactions in a 96-well plate format or 200 assays in 384-well assay format. The kit does not include the enzyme.

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The *S. aureus* DNA Primase Assay Kit Plus (Catalog No. AGA100KE) includes the assay buffer, DNA template, NTP mix and fluorescence dye for 100 assays of DNA primase reactions in a 96-well plate format or 200 assays in 384-well assay format. The kit includes *S. aureus* primase.

The *S. pneumoniae* DNA Primase Assay Kit (Catalog No. PGA100K) includes the assay buffer, DNA template, NTP mix and fluorescence dye for 100 assays of 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 *S. pneumoniae* DNA Primase Assay Kit Plus (Catalog No. PGA100KE) includes the assay buffer, DNA template, NTP mix and fluorescence dye for 100 assays of DNA primase reactions in a 96-well plate format or 200 assays in 384-well assay format. The kit includes *S. pneumoniae* primase.

### Related Products:

DNABG-200EC	<i>E. coli</i> DNA primase-DnaB helicase complex, for 200 assays
DNABG-200HI	<i>H. influenzae</i> DNA primase-DnaB helicase complex, for 200 assays
DNAG-200SA	<i>S. aureus</i> DNA primase, for 200 assays
DNAG-200SP	<i>S. pneumoniae</i> DNA primase, for 200 assays

### Assay Protocol

#### 1. Reagent preparation:

10 x DNA: dilute the 100 x DNA with water.

10 x enzyme: 1  $\mu$ M primase for gram-positive isozyme or 1  $\mu$ M primase plus 0.3  $\mu$ M helicase for gram-negative isozyme. Dilute the 100 x enzyme stock (10  $\mu$ M) with the 1 x assay buffer to make the 10 x enzyme.

10 x NTP mix: dilute the 100 x NTP (a mixture of 50 mM ATP and GTP) 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  $\mu$ l including: 24  $\mu$ l of H<sub>2</sub>O, 4  $\mu$ l of 10 x Buffer, 4  $\mu$ l of 10 x DNA template, 4  $\mu$ l of 10 x enzyme, 4  $\mu$ l of 10 x NTP mix. Incubate the reaction mixture at room temperature for 60 min.

Note: 10 x Buffers: 10 X Buffer EG is for *E. coli* primase; 10 x Buffer HG for *H. influenzae* primase; 10 x Buffer AG for *S. aureus* primase, and 10 x Buffer SG for *S. pneumoniae* primase.

#### 3. Detection:

Add 80  $\mu$ l of the 1 x fluorescence dye into the 40  $\mu$ l of the reaction mixture. Incubate for 5 min. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.