

**ProFoldin Protein Folding Services**

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INSTRUCTIONS

ProFoldin

Bacterial DNA Primase Assay Kits

<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 μ M primase for gram-positive isozyme or 1 μ M primase plus 0.3 μ M helicase for gram-negative isozyme. Dilute the 100 x enzyme stock (10 μ 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 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 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 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.