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INSTRUCTIONS

ProFoldin Bacterial DNA Primase Assay Kits

E. coli DNA Primase Assay Kit

Catalog No. EGA100K

H. influenzae DNA Primase Assay Kit

Catalog No. HGA100K

S. aureus DNA Primase Assay Kit

Catalog No. AGA100K

S. pneumoniae DNA Primase Assay Kit

Catalog No. PGA100K

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 temperate and DNA helicase. The assay can be performed in 96-well plate or 384-well plate format for high throughput screening of DNA primase inhibitors.

Each kit includes the assay buffer, DNA template and fluorescence dye for 100 assays of DNA primase reactions in a 96-well plate format or 200 assays in a 384-well assay format. The following protocol is for the assays in 96-well plates. Please adjust the reagent volumes accordingly for assays in 384-well plates.

Assay Protocol

1. Reagent preparation:

10 x DNA: dilute the 100 x DNA with water

10 x enzyme: 1 μ M primase plus 0.3 μ M helicase (hexamer concentration)

5 mM NTP mix: a mixture of 5 mM ATP, 5 mM GTP, 5 mM CTP and 5 mM UTP

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 5 mM 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.

Note: Fluorescence signals are sensitive to temperature changes. Please keep the temperature consistent during the measurement.