



## ProFoldin

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# INSTRUCTIONS

## ProFoldin

### *E. coli* RNA Polymerase Assay Kits

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*E. coli* RNA Polymerase Assay Kit

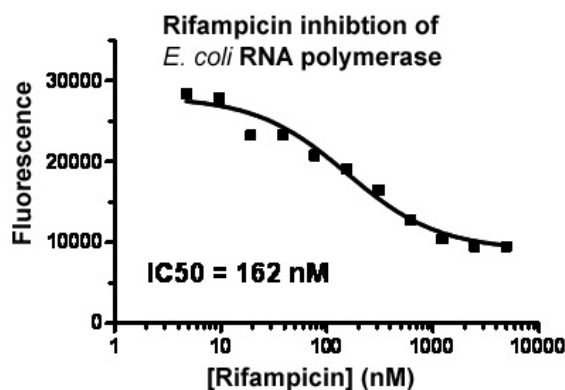
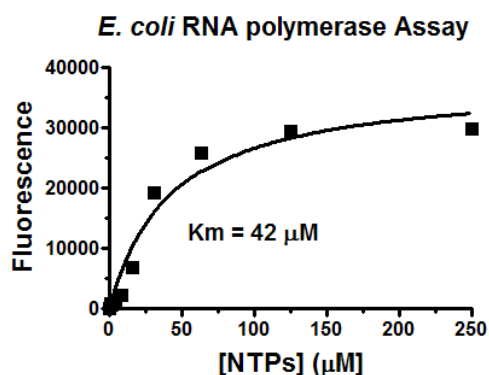
Catalog No. **RPA100K**

*E. coli* RNA Polymerase Assay Kit Plus

Catalog No. **RPA100KE**

### Introduction

The bacterial RNA polymerase is responsible for biosynthesis of mRNA, tRNA and rRNA in the cells. The holoenzyme of bacterial RNA polymerases contain multiple subunits with a molecular mass of about 390 kDa. The bacterial RNA Polymerase Assay Kit is based on measurement of the RNA molecules synthesized by the RNA polymerase using a DNA template. The assay can be performed in 384-well plate format for high throughput screening of RNA polymerase inhibitors.



The *E. coli* RNA Polymerase Assay Kit (Catalog number RPA100K) includes 400 μl of 10 x Buffer, 33 μl 100 x DNA, 33 μl of 100x NTPs, 350 μl of 10 x fluorescence dye. The assay kit includes all reagents except the enzyme. It is for 100 assays of *E. coli* RNA polymerase reactions in a 384-well assay format.

The *E. coli* RNA Polymerase Assay Kit Plus (Catalog number RPA100KE) includes all the reagents in *E. coli* RNA Polymerase Assay Kit (Catalog number RPA100K) plus the enzyme, 33 μl of 100 x *E. coli* RNAP. It is for 100 assays of *E. coli* RNA polymerase reactions in a 384-well assay format.

### Assay Protocol

The following assay protocol is based on the 384-well plate assay format (plate type: Matrix 4318 or alike). The reaction volume is 30 μl and the final assay volume is 60 μl. For 96-well plate assays (plate type: Costar 3915 or alike), the reaction volume is 60 μl and the final assay volume is 120 μl.

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## 1. Reagent preparation:

- (1) 10 x DNA template: dilute the 100 x DNA template 10-fold with water.
- (2) 10 x enzyme: dilute the 100 x RNA polymerase 10-fold with the 1 x Buffer.
- (3) 10 x NTP mix: dilute the 100 x NTP mix (50 mM) 10-fold with water.
- (4) 1 x fluorescence dye: dilute the 10 x fluorescence dye 10-fold with water.

## 2. Reaction:

The total volume of each reaction mixture is 30  $\mu$ l including 18  $\mu$ l of H<sub>2</sub>O, 3  $\mu$ l of 10 x Buffer, 3  $\mu$ l of 10 x DNA template, 3  $\mu$ l of 10 x enzyme, 3  $\mu$ l of 10 x NTP mix. Incubate the reaction mixture in a standard black 384-well plate (Matrix 4318) at 37°C for 60 min.

## 3. Detection:

Add 30  $\mu$ l of the 1 x fluorescence dye into the 30  $\mu$ l of the reaction mixture. Incubate for 5 min. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

## Assay Protocol for enzyme inhibition

The assay can be optimized in terms of assay window, assay linearity and sensitivity to competitive inhibitors. Please contact ProFoldin for HTS assay development service.

## Related Products

### RNA polymerase assay kits:

RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
VRT100K	Viral RNA-dependent RNA Polymerase Assay Kit - 100
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
S2RPA100KE	SARS-CoV-2 RNA polymerase assay kit plus-100

### DNA polymerase assay kits:

DPA100KE	<i>E. coli</i> DNA Polymerase III Alpha Assay Kit Plus
DPA100KH	<i>H influenzae</i> DNA Polymerase Assay Kit Plus
DPA100KN	<i>S. pneumoniae</i> DNA Polymerase Assay Kit Plus
HDP A100KE	Human DNA Polymerase Alpha Assay Kit Plus
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus

For more information of drug targets and enzyme assays, please visit [www.profoldin.com](http://www.profoldin.com) or send emails to [info@profoldin.com](mailto:info@profoldin.com).