



ProFoldin

10 Technology Drive, Suite 40, Number 188
Hudson, MA 01749-2791 USA
Tel: (508)-735-2539 FAX: (508) 845-9258
www.profoldin.com info@profoldin.com

INSTRUCTIONS

ProFoldin *E. coli* RNA Polymerase Assay Kits

E. coli RNA Polymerase Assay Kit

Catalog No. RPA100K

E. coli RNA Polymerase Assay Kit for 1000 Assays

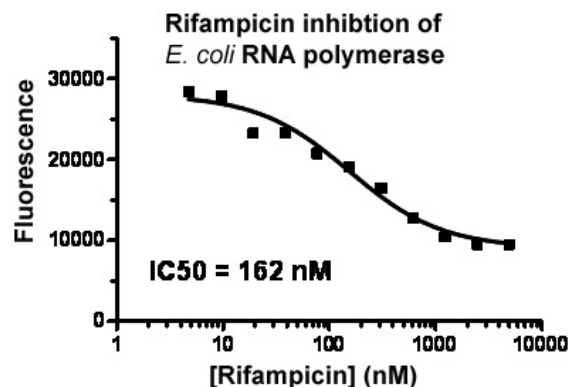
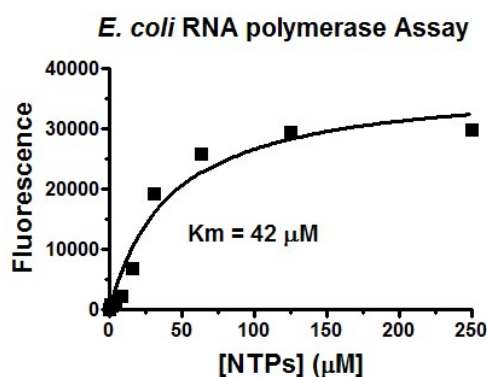
Catalog No. RPA1000K

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 for 1000 Assays (Catalog number RPA1000K) includes 4000 μl of 10 x Buffer, 330 μl 100 x DNA, 330 μl of 100x NTPs, 3500 μl of 10 x fluorescence dye. The assay kit includes all reagents except the enzyme. It is for 1000 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).



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The reaction volume is 30 μ l and the final assay volume is 60 μ l. For 96-well plate assays (plate type: Costar 3915 or alike), the reaction volume is 60 μ l and the final assay volume is 120 μ l.

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 μ l including 18 μ l of H₂O, 3 μ l of 10 x Buffer, 3 μ l of 10 x DNA template, 3 μ l of 10 x enzyme, 3 μ 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 μ l of the 1 x fluorescence dye into the 30 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 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

RPA-100EC	<i>E. coli</i> RNA Polymerase – for 100 assays
RPA-1000EC	<i>E. coli</i> RNA Polymerase – for 1000 assays
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
RPAP100KE	<i>E. coli</i> RNA Polymerase Assay Pyrophosphate Kit Plus
RPAP100KSE	<i>S. aureus</i> RNA Polymerase Assay Pyrophosphate Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus
DPA100KE	<i>E. coli</i> DNA Polymerase III Alpha Assay Kit Plus
HDPA100K	Human DNA Polymerase Alpha Assay Kit
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
PAR100KE	Human Poly (ADP-ribose) Polymerase-1 Assay Kit Plus

For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.