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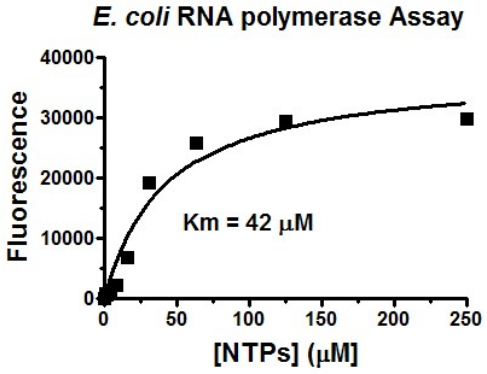
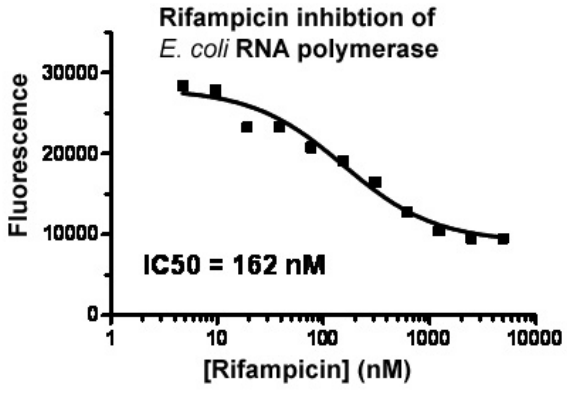
INSTRUCTIONS

ProFoldin
E. coli RNA Polymerase

***E. coli* RNA Polymerase – for 100 assays** **Catalog No. RNAP-100EC**

Protein construct:	Wild-type *E.coli* RNA Polymerase purified from *E. coli.*
MW:	389 kDa
Enzyme concentration:	2 μM
RNA polymerase assay:	The RNA polymerase activity is measured by using the RNA Polymerase Assay kit (Catalog No. RPA100K).
Storage temperature:	-20 or -80°C. Do not freeze-and-thaw repeatedly.
Enzyme dilution:	Use the 1 x assay to dilute the enzyme just before the assay. Do not store diluted enzyme solution.





The ***E.coli* RNA Polymerase –for 100 assays (Catalog No. RPA-100EC**) includes 33 μl of 100 x *E.coli* RNA Polymerase (2 μM). It is for 100 assays.

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.

1. Reagent preparation:

(1) 10 x DNA template: dilute the 100 x DNA template10-fold with water.

(2) 10 x enzyme: dilute the 100 x RNA polymerase 10-fold with the 1 x Buffer.

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(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 µl including 18 µl of H_2O, 3 µl of 10 x Buffer, 3 µl of 10 x DNA template, 3 µl of 10 x enzyme, 3 µl 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 µl of the 1 x fluorescence dye into the 30 µl 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

RPA100K	*E. coli* RNA Polymerase Assay Kit
RPA1000K	*E. coli* RNA Polymerase Assay Kit for 1000 Assays
RPA100KE	*E. coli* RNA Polymerase Assay Kit Plus
RPA100KSE	*S. aureus* RNA Polymerase Assay Kit Plus
RPAP100KE	*E. coli* RNA Polymerase Assay Pyrophosphate Kit Plus
RPAP100KSE	*S. aureus* 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	*E. coli* 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

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