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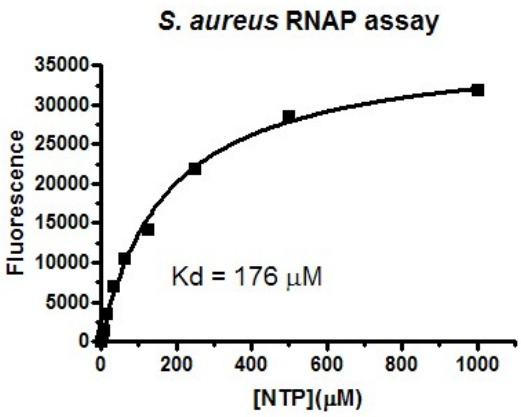
INSTRUCTIONS

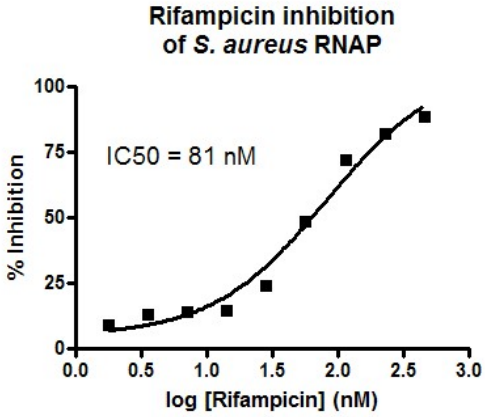
ProFoldin
S. aureus RNA Polymerase Assay Kits

***S. aureus* RNA Polymerase Assay Kit** **Catalog No. RPA100KS**
***S. aureus* RNA Polymerase Assay Kit Plus** **Catalog No. RPA100KSE**

Introduction

The bacterial RNA polymerase is responsible for biosynthesis of mRNA, tRNA and rRMA in the cells. The holoenzyme of bacterial RNA polymerases contain multiple subunits with a molecular mass of about 400 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 ***S. aureus* RNA Polymerase Assay Kit** (Catalog number RPA100KS) includes 400 µl 10 x Buffer, 33 µl 100 x DNA, 33 µl 100x NTPs, 350 µl 10 x fluorescence dye. The assay kit includes all reagents except the enzyme. It is for 100 assays of *S. aureus* RNA polymerase reactions in a 384-well assay format.

The ***S. aureus* RNA Polymerase Assay Kit Plus** (Catalog number RPA100KSE) includes all the reagents in ***S. aureus* RNA Polymerase Assay Kit** (Catalog number RPA100KS) plus the enzyme, 33 µl 100 x *S. aureus* RNAP. It is for 100 assays of *S. aureus* RNA polymerase reactions in a 384-well assay format.

Reference:

Siricilla S. *et al,* Discovery of a capuramycin analog that kills nonreplicating Mycobacterium tuberculosis and its synergistic effects with translocase I inhibitors. *The Journal of Antibiotics,* 68, 271–278 (2015).

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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.
(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.

Note: The final concentration of *S. aureus* RNA polymerase in the assay is 20 nM. The assay buffer is 42.5 mM HEPES, pH 7.5, 42.5 mM NH_4Cl, 2 mM DTT, 4 mM $MgCl_2$, 0.005% Tween-20.

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. ProFoldin offers HTS assay development service. For more information, please visit our website at http://www.profoldin.com/services.html.

Related Products

RPAP100KSE	*S. aureus* RNA Polymerase Assay Pyrophosphate Kit Plus
RPA100KE	*E. coli* RNA Polymerase Assay Kit Plus
RPAP100KE	*E. coli* RNA Polymerase Assay Pyrophosphate Kit Plus
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
HIV100K	HIV Reverse Transcriptase Assay Kit
MLV100K	M-MLV Reverse Transcriptase Assay Kit
AMV100K	AMV Reverse Transcriptase Assay Kit
VRT100K	Viral RNA-dependent RNA Polymerase Assay Kit
DPA100KE	*E. coli* DNA Polymerase III Alpha Assay Kit Plus
HDPA100K	Human DNA Polymerase Alpha Assay Kit
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus

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