



ProFoldin

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INSTRUCTIONS

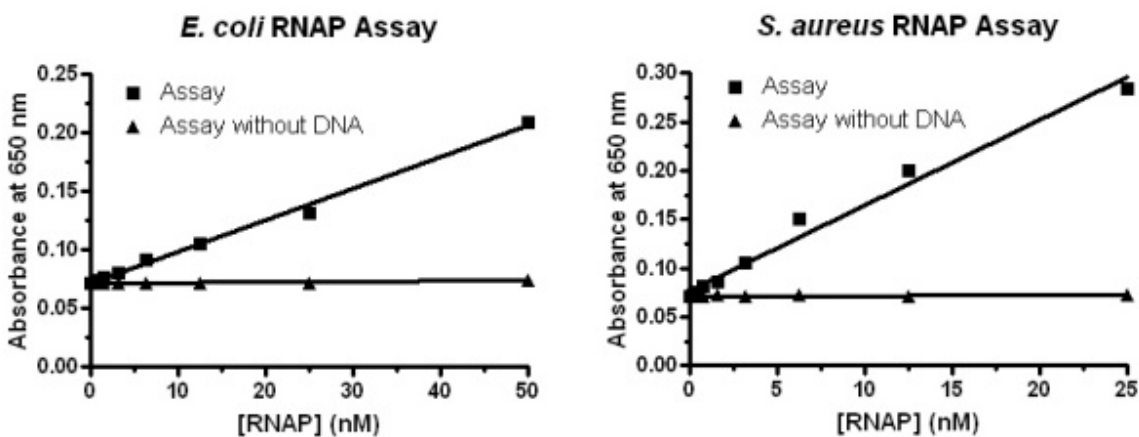
ProFoldin Bacterial RNA Polymerase Pyrophosphate Assay Kits

Bacterial RNA Polymerase Pyrophosphate Assay Kit
***E. coli* RNA Polymerase Pyrophosphate Assay Kit Plus**
***S. aureus* RNA Polymerase Pyrophosphate Assay Kit Plus**
Pyrophosphate Assay Reagent for RNAP

Catalog No. RPAP100K
Catalog No. RPAP100KE
Catalog No. RPAP100KSE
Catalog No. PPA1000

Introduction

The bacterial RNA polymerase is responsible for biosynthesis of mRNA, tRNA and rRNA in the cells. The holoenzyme of bacterial RNA polymerases contains multiple subunits with a molecular mass of about 400 kDa. The bacterial RNA Polymerase Pyrophosphate Assay Kit is based on measurement of the pyrophosphate generated in the RNA polymerase reaction using a double strand DNA template. The pyrophosphate is converted to phosphate by pyrophosphatase and the phosphate is detected by the phosphate detection reagent (catalog number MPA3000).



The assay can be performed in a 384-well plate format for high throughput screening of RNA polymerase inhibitors. For assay kits measuring RNA formation, please see Related Products below.

The **Bacterial RNA Polymerase Pyrophosphate Assay Kit** (Catalog number RPAP100K) includes 400 μ l of Buffer RP, 33 μ l of 100 x DNA, 33 μ l of 100 x NTP mix, 10 μ l of 1000 x pyrophosphatase and 4500 μ l of dye for 100 assays of *E. coli* RNA polymerase reactions in a 384-well assay format. The assay kit includes all reagents except the enzyme.

The ***E. coli* RNA Polymerase Assay Pyrophosphate Kit Plus** (Catalog number RPAP100KE) includes 400 μ l of Buffer RP, 33 μ l of 100 x DNA, 33 μ l of 100 x NTP mix, 10 μ l of 1000 x pyrophosphatase, 33 μ l of 100 x *E. coli*



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RNA polymerase and 4500 μ l of dye for 100 assays of *E. coli* RNA polymerase reactions in a 384-well assay format.

The ***S. aureus* RNA Polymerase Assay Pyrophosphate Kit Plus** (Catalog number RPAP100KSE) includes 400 μ l of Buffer RP, 33 μ l of 100 x DNA, 33 μ l of 100 x NTP mix, 10 μ l of 1000 x pyrophosphatase, 33 μ l of 100 x *S. aureus* RNA polymerase and 4500 μ l of dye for 100 assays of *S. aureus* RNA polymerase reactions in a 384-well assay format.

The **Pyrophosphate Assay Reagent for RNAP** (Catalog number PPA1000) includes 3200 μ l of Buffer RP, 310 μ l of 100 x NTP mix, 310 μ l of 100 x DNA, 32 μ l of 1000 x pyrophosphatase and 42 ml of dye for 100 assays of RNA polymerase reactions in a 384-well assay format. The assay kit includes all reagents except the enzyme.

Assay Protocol

1. Reagent preparation:

1x assay buffer: dilute the 10 x Buffer RP 10-fold with water.

10 x DNA: dilute the 100 x DNA 10-fold with water.

10 x enzyme: dilute the 100 x RNA polymerase (Catalog #: RNAP-100EC) 10-fold with the 1 x assay buffer.

10 x NTP mix: dilute the 100 x NTP mix 10-fold with water.

10 x pyrophosphatase: dilute the 1000 x pyrophosphatase (100 U/ml) 100-fold with the 1 x assay buffer.

2. Reaction:

A transparent 384-well plate (Matrix 4310, for example) is used for the assay. The total volume of each reaction mixture is 30 μ l including 15 μ l of H₂O, 3 μ l of 10 x Buffer RP, 3 μ l of 10 x DNA template, 3 μ l of 10 x enzyme, 3 μ l of 10 x pyrophosphatase, 3 μ l of 10 x NTP mix. Incubate the reaction mixture at 37°C for 60 min.

3. Detection:

Add 40 μ l of the dye (MPA3000) into the 30 μ l of the reaction mixture. Incubate for 3 min. Measure the light absorbance at 650 nm.

Related Products

RNAP-100EC	<i>E. coli</i> RNA polymerase - for 100 assays
RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus (detecting RNA formation)
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus (detecting RNA formation)
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus
VRT100K	Viral RNA-dependent RNA Polymerase Assay Kit - 100

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