



## ProFoldin

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# INSTRUCTIONS

## ProFoldin T7 RNA Polymerase Assay Kit

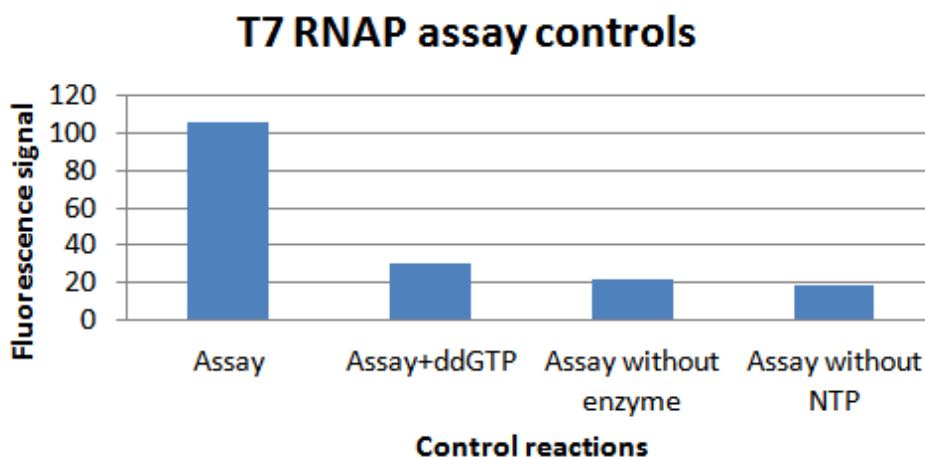
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**T7 RNA Polymerase Assay Kit**  
**T7 RNA Polymerase Assay Kit Plus**

**Catalog No. T7RPA100K**  
**Catalog No. T7RPA100KE**

### Introduction

The assay for bacteriophage T7 RNA polymerase is based on measurement of the RNA molecules synthesized by the RNA polymerase using fluorescence detection. The assay can be performed in a 384-well or 96-well plate format for tests of T7 RNA polymerase activities and high throughput screening of T7 RNA polymerase inhibitors.



The **T7 RNA Polymerase Assay Kit** (Catalog number T7RPA100K) includes 400  $\mu$ l of 10 x Buffer, 33  $\mu$ l of 100 x DNA, 33  $\mu$ l of 100 x NTPs, 1550  $\mu$ l of 2 x Dye and 1550  $\mu$ l of 50 mM EDTA. It is for 100 assays of T7 RNA polymerase. The assay kit includes all reagents except the enzyme.

The **T7 RNA Polymerase Assay Kit Plus** (Catalog number T7RPA100KE) includes all reagents in **T7 RNA Polymerase Assay Kit** (Catalog number T7RPA100K) plus the enzyme, 33  $\mu$ l 100 x T7 RNA polymerase.

### Publications

Siegmund V. et al, Screening mutant libraries of T7 RNA polymerase for candidates with increased acceptance of 2'-modified nucleotides, Chem. Commun., 48, 9870-9872 (2012).

### 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  $\mu$ l and the final assay volume is 60  $\mu$ l. For 96-well plate assays (plate type: Costar 3915 or alike), the reaction volume is 60  $\mu$ l and the final assay volume is 120  $\mu$ l.

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## 1. Reagent preparation:

- (1) 10 x DNA: Dilute the 100 x DNA 10-fold with water. Each assay uses 3  $\mu$ l of 10 x DNA.
- (2) 10 x enzyme: Dilute the 100 x T7 RNA polymerase (50 Unit /  $\mu$ l) 10-fold with the 1 x assay buffer. Each assay uses 3  $\mu$ l of 10 x enzyme.
- (3) 10 x NTP mix: Dilute the 100 x NTP mix (50 mM) 10-fold with water. Each assay uses 3  $\mu$ l of 10 x NTP mix.
- (4) 1 x dye: Dilute the 2 x fluorescence dye 2-fold with 50 mM EDTA. Each assay uses 30  $\mu$ l of 1 x dye.

## 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 at 37°C for 60 min.

## 3. Detection:

Mix 30  $\mu$ l of the 1 x fluorescence dye with 30  $\mu$ l of the reaction mixture. 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

RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
MRPA100K	Human Mitochondrial RNA Polymerase Assay Kit
AMV100K	AMV Reverse Transcriptase Assay Kit
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
HIV100K	HIV Reverse Transcriptase Assay Kit
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
MLV100K	M-MLV Reverse Transcriptase Assay Kit
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
DPB100K	Human DNA Polymerase Beta Assay Kit
DPG100K	Human DNA Polymerase Gamma Assay Kit
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus

## More information of drug targets and enzyme assays

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).