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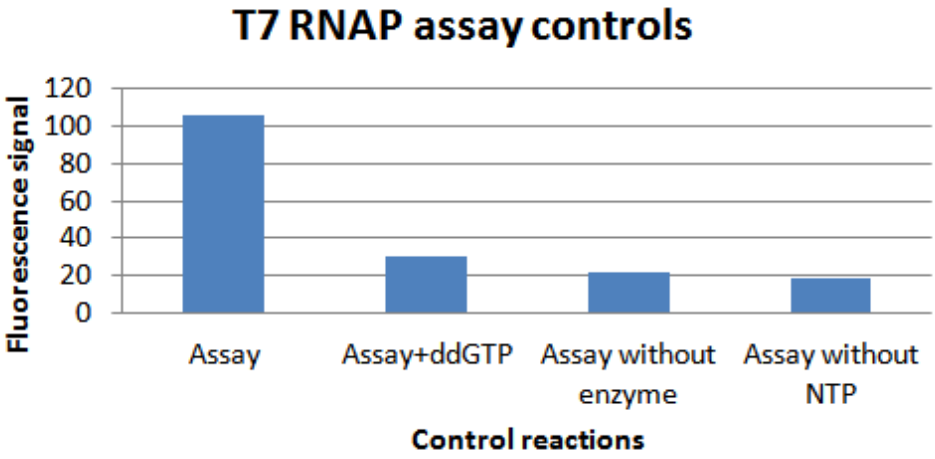
INSTRUCTIONS

ProFoldin
T7 RNA Polymerase Assay Kit

T7 RNA Polymerase Assay Kit **Catalog No. T7RPA100K**
T7 RNA Polymerase Assay Kit Plus **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 µl of 10 x Buffer, 33 µl of 100 x DNA, 33 µl of 100 x NTPs and 610 µl of 5 x Dye. 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 µl 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 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.

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1. **Reagent preparation:**
   (1) 10 x DNA: Dilute the 100 x DNA 10-fold with water. Each assay uses 3 µl of 10 x DNA.
   (2) 10 x enzyme: Dilute the 100 x T7 RNA polymerase (50 Unit / µl) 10-fold with the 1 x assay buffer. Each assay uses 3 µl 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 µl of 10 x NTP mix.
   (4) 1 x dye: Dilute the 5 x fluorescence dye 5-fold with water. Each assay uses 30 µl of 1 x dye.

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 at 37°C for 60 min.

3. **Detection**:
   Mix 30 µl of the 1 x fluorescence dye with 30 µl 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

RNA polymerase assay kits:

S2RPA100KE	SARS-CoV-2 RNA polymerase (RdRp) assay kit plus -100
ZRPA100KE	Zika Virus RNA-dependent RNA Polymerase Assay Kit Plus-100
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus
RPA100KE	*E. coli* RNA Polymerase Assay Kit Plus
RPA100KSE	S. *aureus* RNA Polymerase Assay Kit Plus
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
EGA100KE	*E. coli* DNA Primase Assay Kit Plus

DNA polymerase assay kits:

DPA100KE	*E. coli* DNA Polymerase III Alpha Assay Kit Plus
DPA100KH	*H influenzae* DNA Polymerase Assay Kit Plus
DPA100KN	*S. pneumoniae* DNA Polymerase Assay Kit Plus
HDPA100KE	Human DNA Polymerase Alpha Assay Kit Plus
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus
HDPQ100KE	Human DNA Polymerase Theta ATPase Assay Kit Plus

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