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INSTRUCTIONS

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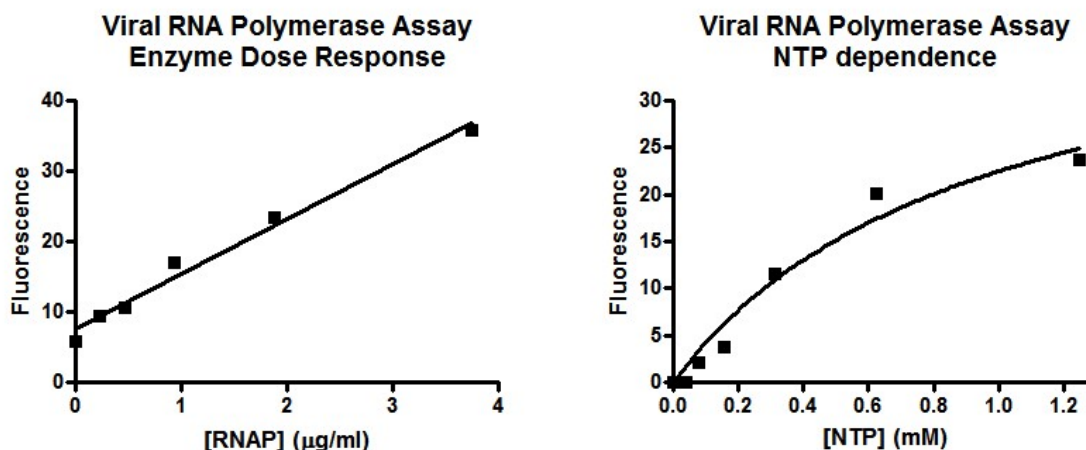
Viral (Flavivirus) RNA-dependent RNA Polymerase Assay Kits

Viral (Flavivirus) RNA-dependent RNA Polymerase Assay Kit - 100 Catalog No.VRT100K

Viral (Flavivirus) RNA-dependent RNA Polymerase Assay Kit - 500 Catalog No.VRT500K

Introduction

The Viral RNA-dependent RNA Polymerase Assay is developed using a RNA polymerase in the *Flaviviridae*, a family of positive, single-stranded, enveloped RNA viruses. The assay is based on measurement of the RNA molecules synthesized by the RNA polymerase using RNA as a template in the presence of NTPs. The assay can be performed in a 384-well or 96-well plate format for tests of the enzyme activities of RNA polymerases in the *Flaviviridae* family and high throughput screening of inhibitors.



The **Viral (Flavivirus) RNA-dependent RNA Polymerase Assay Kit – 100** (Catalog No.VRT100K) includes 350 µl of 10 x Buffer, 350 µl of 10 x MnCl₂, 33 µl of 100 x Template, 33 µl of 100 x NTPs (50 mM ATP and 50 mM GTP) and 330 µl of 10 x fluorescence dye. It is for 100 assays of virus RNA polymerase in a 384-well plate format. The assay kit includes all reagents except the enzyme.

The **Viral (Flavivirus) RNA-dependent RNA Polymerase Assay Kit – 500** (Catalog No.VRT500K) includes 1550 µl of 10 x Buffer, 1550 µl of 10 x MnCl₂, 160 µl of 100 x Template, 160 µl of 100 x NTPs (50 mM ATP and 50 mM GTP) and 1550 µl of 10 x fluorescence dye. It is for 500 assays of virus RNA polymerase in a 384-well plate format. The assay kit includes all reagents except the enzyme.

Reference

Shu Yang et al, Emetine inhibits Zika and Ebola virus infections through two molecular mechanisms: inhibiting viral replication and decreasing viral entry, *Cell Discovery*, Volume 4, Article number: 31, (05 June 2018).



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

1. Reaction:

The total volume of each reaction mixture is 30 μ l including 23.1 μ l of H₂O, 3 μ l of 10 x Buffer, 3 μ l of 10 x MnCl₂, 0.3 μ l of 100 x template, 0.3 μ l of 100 x RNA polymerase and 0.3 μ l of 100 x NTPs. Incubate the reaction mixture at 37°C for 60 min.

2. Detection:

Dilute the 10x fluorescence dye with water to make the 1 x dye. Mix 30 μ l of the 1 x dye with 30 μ l of the reaction mixture. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm in 5 min.

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:

S2RPA100K	SARS-CoV-2 RNA polymerase assay kit-100
RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
EGA100KE	<i>E. coli</i> DNA Primase Assay Kit Plus
AGA100KE	<i>S. aureus</i> DNA Primase Assay Kit Plus
PGA100KE	<i>S. pneumoniae</i> DNA Primase Assay Kit Plus

DNA polymerase assay kits:

DPA100KE	<i>E. coli</i> DNA Polymerase III Alpha Assay Kit Plus
DPA100KH	<i>H influenzae</i> DNA Polymerase Assay Kit Plus
DPA100KN	<i>S. pneumoniae</i> DNA Polymerase Assay Kit Plus
HDP A100KE	Human DNA Polymerase Alpha Assay Kit Plus
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus

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