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10 Technology Drive, Suite 40, Number 188
Hudson, MA 01749-2791 USA
FAX: (508) 845-9258 Tel: (508) 735-2539
www.profoldin.com
info@profoldin.com

INSTRUCTIONS

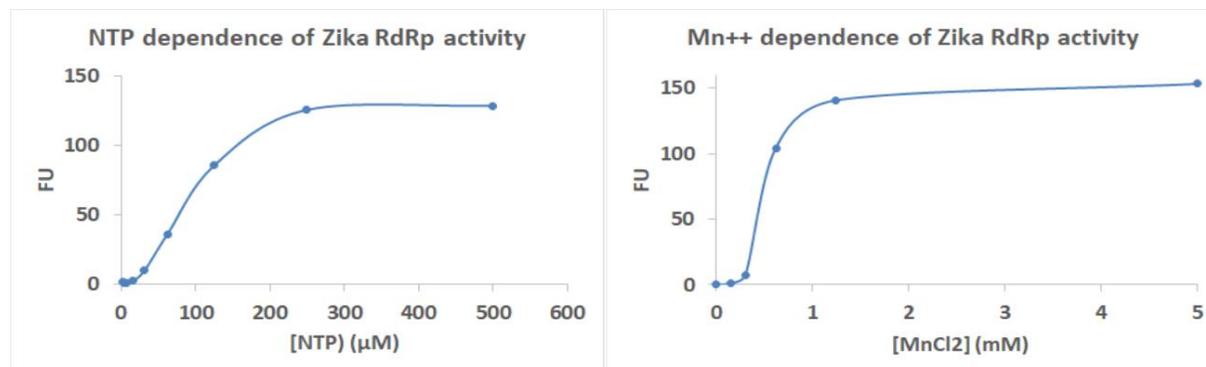
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Viral (Flavivirus) RNA-dependent RNA Polymerase Assay Kits

Zika Virus RNA-dependent RNA Polymerase Assay Kit Plus-100	Catalog No. ZRPA100KE
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

Zika virus is a positive, single-stranded, enveloped RNA virus. Zika virus belongs to the Flaviviridae family. Zika virus infection transmitted by mosquitoes is a trigger of Guillain-Barré syndrome, neuropathy and myelitis. Infection in pregnancy results in pregnancy complications such as fetal loss, stillbirth and preterm birth. The RNA-dependent RNA Polymerase (RdRp) is an essential enzyme for the virus and is an attractive drug target. The viral RdRp assay is based on detection of RNA synthesis by the RdRp enzyme. The assay can be performed in a 384-well or 96-well plate format for tests of the enzyme activities of Zika RNA polymerase and high throughput screening of inhibitors.



The **Zika Virus RNA-dependent RNA Polymerase Assay Kit Plus-100** (Catalog No. ZRPA100KE) includes 350 μl of 10 x Buffer, 350 μl of 10 x MnCl₂, 33 μl of 100 x Template, 33 μl of 100 x NTP, 31 μl of 100 x Zika virus RdRp and 330 μl of 10 x fluorescence dye. It is for 100 assays of virus RNA polymerase in a 384-well plate format.

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 NTP 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



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NTPs and 1550 μ l 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.

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 50 μ l and the final assay volume is 100 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 30°C for 90 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.

Reference

1. 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).
2. Chen Y. et al, Cinnamic acid inhibits Zika virus by inhibiting RdRp activity. *Antiviral Research* Volume 192, August 2021, 105117.

Related Products

RNA polymerase assay kits:

S2RPA100KE	SARS-CoV-2 RNA polymerase assay kit plus -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

DNA polymerase assay kits:

DPA100KE	<i>E. coli</i> DNA Polymerase III Alpha 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.