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INSTRUCTIONS

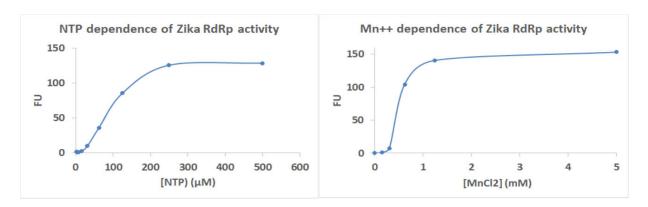
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Viral (Flavivirus) RNA-dependent RNA Polymerase Assay Kits

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

The *Flaviviridae* virus family are positive, single-stranded, enveloped RNA viruses. Zika virus is one of the *Flaviviridae* family. 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 RNA polymerases in the *Flaviviridae* family and high throughput screening of inhibitors.



The Zika Virus RNA-dependent RNA Polymerase Assay Kit Plus-100 (Catalog No. ZRPA100KE) includes 350 μ l of 10 x Buffer, 350 μ l of 10 x MnCl₂, 33 μ l of 100 x Template, 33 μ l of 100 x NTP, 31 μ l of 100 x Zika virus RdRp and 330 μ l 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 μ l of 10 x Buffer, 350 μ l of 10 x MnCl₂, 33 μ l of 100 x Template, 33 μ l of 100 x NTP and 330 μ l 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 μ l of 10 x Buffer, 1550 μ l of 10 x MnCl₂, 160 μ l of 100 x Template, 160 μ l of 100 x 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.

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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 50 μ l and the final assay volume is 100 μ l.

1. Reaction:

The total volume of each reaction mixture is 30 μl including 23.1 μl of H₂O, 3 μl of 10 x Buffer, 3 μl of 10 x MnCl₂, 0.3 μl of 100 x template, 0.3 μl of 100 x RNA polymerase and 0.3 μl 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 1x dye. Mix $30 \mu l$ of the 1x dye with $30 \mu l$ 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

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MRPA100KE Human Mitochondrial RNA Polymerase Assay Kit Plus

T7RPA100KE T7 RNA Polymerase Assay Kit Plus EGA100KE E. coli DNA Primase Assay Kit Plus

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