ProFoldin 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

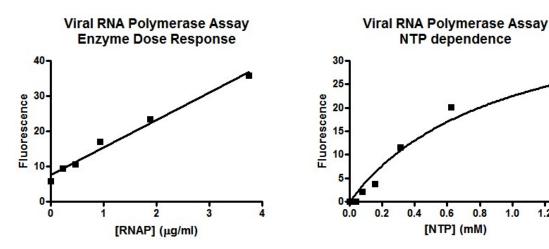
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Viral RNA-dependent RNA Polymerase Assay Kits

Viral RNA-dependent RNA Polymerase Assay Kit - 100 Catalog No.VRT100K Viral 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 RNA-dependent RNA Polymerase Assay Kit -100 (Catalog No.VRT100K) includes 350 μ l of 10 x Buffer, 33 μ l of 100 x Template, 33 μ l of 100 x NTPs (50 mM ATP and 50 mM GTP) 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 RNA-dependent RNA Polymerase Assay Kit** - **500** (Catalog No.VRT500K) includes 1550 μ l of 10 x Buffer, 160 μ l of 100 x Template, 160 μ l of 100 x NTPs (50 mM ATP and 50 mM GTP) 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.

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 26.1 μ l of H₂O, 3 μ l of 10 x Buffer, 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 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.

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.

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