Virongy Biosciences
Accelerate Discovery

Hybrid-Alpha Nipah Virus (Ha-NiV)

Utilizing the proprietary rapid-expressing hybrid-alphavirus

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1. Introduction

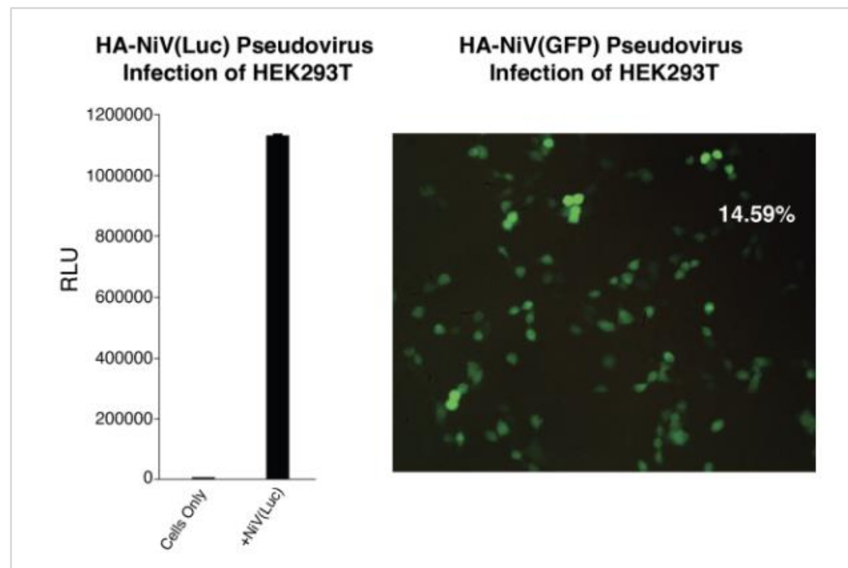
Ha-NiV is a hybrid-alpha pseudovirus derived from Virongy's proprietary alphavirus vector genome. Unlike lenti- or vesicular stomatitis virus (VSV) pseudoviruses, which typically express only F and G proteins, Ha-NiV contains four structural Nipah Virus proteins: Fusion (F), Glycoprotein (G), Matrix (M), and Nucleocapsid (N) to better simulate entry of the live virus. Ha-NiV is assembled using Virongy's alpha-pseudovirus genome. The particle is a single-cycle virus with self-replicating RNA, allowing for rapid quantification of neutralizing antibodies and entry-inhibiting drugs.

Key Features

- Rapid assay results in 6 hours.
- Expression of four NiV structural proteins (F, G, M and N)
- Contains a reporter genome derived from an alphavirus-based vector for rapid and robust expression of reporter genes
- High reproducibility and consistency between sample infections

Applications

- Rapid NiV pseudovirus transduction of target cells for viral entry and functional studies
- Anti-NiV drug screening
- Anti-NiV neutralizing antibody screening



(**Left**) Ha-NiV particles were assembled with the NiV F, G, M, and N proteins and Virongy's proprietary alphavirus vector genome. The Ha-NiV pseudovirus carrying the luciferase reporter gene was used to transduce HEK293T cells and reporter expression was quantified 24 hrs post-transduction. (**Right**) The Ha-NiV pseudovirus carrying the GFP reporter gene was used to transduce HEK293T cells and GFP expression was quantified 24 hrs post-transduction.

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2. Product Information

This product has been manufactured for research use only. This product has not been developed for the treatment or diagnosis of disease in humans or animals.

THE PRODUCT IS INTENDED FOR USE ONLY BY PROFFESIONALS WHO HAVE BEEN TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES.

Storage

Product is shipped frozen and on dry ice. Upon receiving, store at -80 °C.

Safety Information

Please find the Material Safety Data Sheet (MSDS) associated with this product at www.virongy.com.

3. Infection Protocol:

*See Table 1 below for scaling information

1. Seed cells in sample wells and allow at least 1hr for cells to adhere.
2. Once cells have adhered, completely thaw Ha-NiV particles and add to the wells.
3. Incubate the plate at 37°C for a minimum of 6 hours. For higher infectivity, incubate the plate overnight.
4. For Ha-NiV(GFP) pseudovirus, GFP can be observed and quantified directly with microscopy or flow cytometry.
5. For Ha-NiV(Luc) pseudovirus, following the protocol below for luciferase assay:

Table 1: Infection Scaling

Infection Plate	Cell Number	Cell Volume	Virus Volume
96-well	5×10^4	30 µL	45 µL
12-well	2×10^5	300 µL	200 µL
6-well	6×10^5	2 mL	400 µL
10 cm dish	$3.5\text{-}5 \times 10^6$	10 mL	1 mL

4. Luciferase Assay Protocol for 96-well Plate

Before starting: Thaw the **10X Cell Lysis Buffer**, **Firefly Luciferase Buffer**, and the **D-Luciferin** in the biosafety cabinet before preparing the reagent mixtures. Keep all reagents on ice once thawed. Turn off the lights in the vicinity of the luminometer before loading the plate.

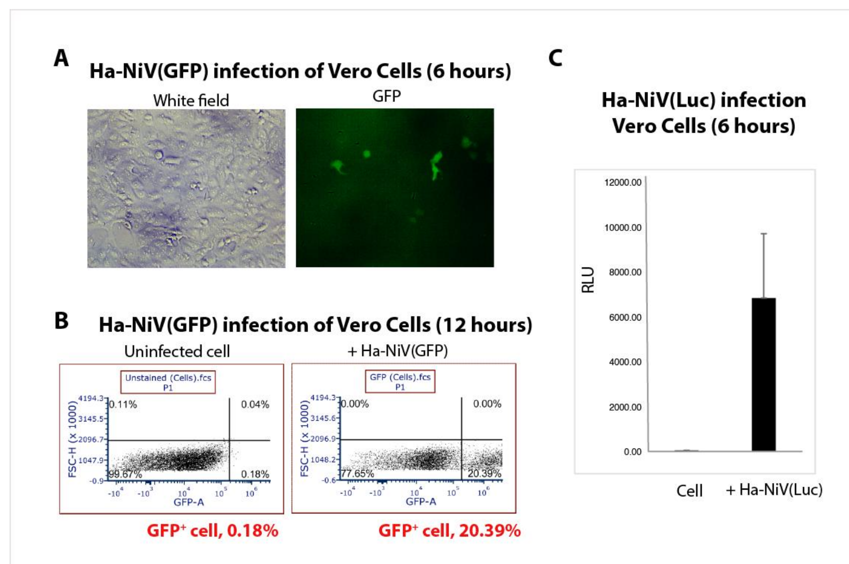
1. Following the incubation period, remove the medium from each well of the infection plate.
2. Wash each well with 200 µL of PBS.
3. Lyse the cells with 35 µL 1X cell lysis buffer. Allow the cells to lyse for at least 5 minutes at room temperature.
4. In a dark location, away from direct light, prepare the Firefly Luciferase Assay Solution by mixing D-Luciferin with Firefly Luciferase Buffer in a 1:50 ratio. For a full 96-well plate prepare 3 mL of the luciferase substrate solution by mixing 60 µL of the D-Luciferin with 2940 µL of the Firefly Luciferase Buffer. (Note: Use the Firefly Luciferase Assay Solution within 30 minutes of preparation. Do not reuse the Firefly Luciferase Assay Solution.)

5. Add 25 µL of Firefly Luciferase Assay Solution into each well with cell lysates.
6. The plate should be analyzed in a luminometer within 10-15 minutes of adding the Firefly Luciferase Assay Solution.

5. Further Information

- Material Safety Data Sheet (MSDS): www.virongy.com
- Please find the most up-to-date version of this handbook at www.virongy.com
- Technical assistance: info@virongy.com

Example results:



(**A**) Ha-NiV(GFP) particles were used to infect Vero cells and GFP expression was observed at 6 hrs post-transduction. (**B**) GFP expression was also quantified at 12 hrs post-transduction by flow cytometry. (**C**) Ha-NiV(Luc) particles were used to infect Vero cells and Luc expression was quantified at 6 hrs post-transduction.