

**Influenza H3 Pseudovirus
A/Hong Kong/1/1968 (H3N2) strain**

Luciferase reporter

Lot #251215 - H3HK



Certificate of Analysis

1. Summary

This certificate is a functional validation for the lot #251215 - H3HK of an avian HA pseudotyped pseudovirus, A/Hong Kong/1/1968 (H3N2) strain. The titer is 3.7×10^5 RLU / μL . A volume of 1 mL can be used to perform 1,000 reactions or 10 x 96-well plates, according to IVANO Bioscience’s protocol available upon request.

2. Transduction efficiency assay

Target cells HEK293T cells
Volume of pseudovirus 0 – 0.5 – 1 – 2 – 4 – 6 – 8 – 10 $\mu\text{L}/\text{well}$
Detection signal Luminescence (firefly luciferase)
Detection method Microplate reader Biotek Synergy H1 (Gain: 135)

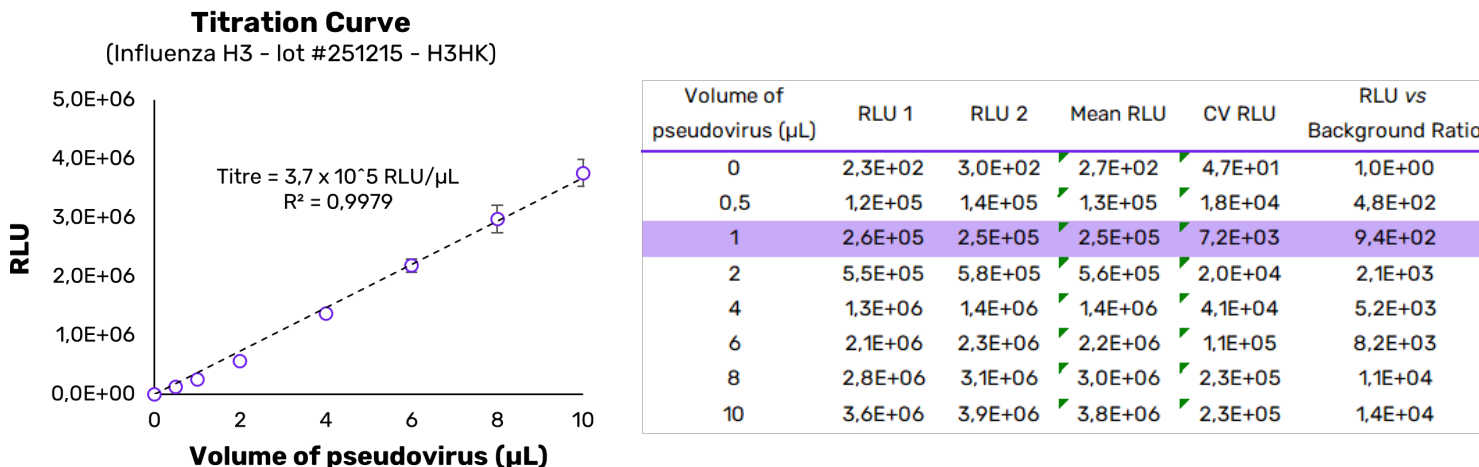


Figure 1: Transduction efficiency curve

A volume range of pseudovirus was mixed in a final volume of 50 μL of medium, in a 96-well plate. Then, 50 μL of medium containing 10,000 cells was seeded in each well. On the day of analysis, an additional 100 μL of Bright-Glo™ Luciferase reagent was added in each well and incubated for 2 minutes. Data in relative unit luminescence (RLU) were obtained from the analysis of 150 μL of the cell lysate, using a microplate reader. Data are expressed in relative unit luminescence (RLU).

Conclusion

The Influenza H3 pseudovirus (#251215 - H3HK) can transduce the target cells. The titer is 3.7×10^5 RLU/ μL . Using 1 $\mu\text{L}/\text{reaction}$ of pseudovirus in a 96-well plate will yield a 1,000-fold increase in RLU compared to the background. Therefore, 1 mL of lot #251215 - H3HK could be used to perform approximately 1,000 reactions or 10 x 96-well plates, according to IVANO Bioscience’s protocol (available upon request).

3. Neutralization assay

Target cells	HEK293T cells
Volume of pseudovirus	1 μ L / well
Neutralizing antibody (Nabs)	Anti-H3 surface glycoprotein - In-house antibody
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)

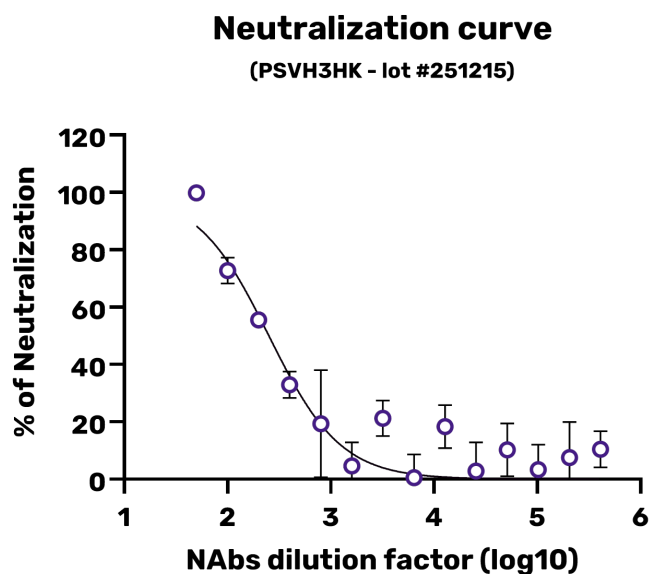


Figure 2: Neutralization curve

A starting dilution of 1/100 of a neutralizing antibody was serially diluted in a final volume of 50 μ L of medium and incubated for 1 hour at 37 $^{\circ}$ C, with 1 μ L of pseudovirus, in a 96-well plate. Then, 50 μ L of medium, containing 10,000 cells, was seeded in each well and incubated for 72 hours. On the day of analysis, an additional 100 μ L of Bright-Glo™ Luciferase reagent was added in each well and incubated for 2 minutes. Data in relative unit luminescence (RLU) were obtained from the analysis of 150 μ L of the cell lysate, using a microplate reader. Raw data were analyzed using a log(inhibitor) vs normalized-response (variable slope) non-linear regression model in Prism v10 (GraphPad). Percentages of neutralization were normalized considering only cells into wells as 100% neutralization and cells transduced by pseudoviruses without any NABs as 0% neutralization. Data are representative of duplicates.

Conclusion

The Influenza H3 pseudovirus (#251215 - H3HK) can be efficiently neutralized by neutralizing antibodies.

4. Additional information

Intruction of use

We recommend determining the titer in your lab's conditions before performing any experiments.

Handle under biosafety level-2.

Pseudovirus

3rd generation lentiviral vector, incompetent replication and non-toxic.

Pseudotyping

Influenza hemagglutinin, strain A/Hong Kong/1/1968 (H3N2) (Gen-Bank: AAK51719.1)

Pseudotyping sequence

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MNTQILVFALIAIIPANADKICLGHHAVSNGTKVNTLTERGVEVVNATETVERTNIP  
RICKSGKRTVDLGGQCGLLGTITGPPQCDQFLEFSADLIERREGSDVCYPGKFDV  
EEALRQILRESGGIDKEAMGFTYSGIRTNGATSACRRSGSSFYAEMKWLLSNTD  
NAAFPQMTKSYKNTRKSPALIVWGIHHSVSTAEQTKLYGSGNKLVTVGSSNYQ  
QSFVPSPGARPQVNGLSGRIDFWLMLNPNDTVTFSFNGAFIAPDRASFLRGKS  
MGIQSGVQVDANCEGDCHHSGGTIISNLPFQNIDSRVAVGKCPRYVKQRSLLAT  
GMKNVPEIPKGRGLFGAIAAGFIENGWEGLIDGWYGFRHQNAQGEFTAADYKST  
QSAIDQITGKLNRLIEKTNQQFELIDNEFNEVEKQIGNVINWTRDSITEVWSYNAE  
LLVAMENQHTIDLADSEMDKLYERVKRLRENAEEDGTGCFEIFHKCDDDCMA  
SIRNNTYDHSKYREEAMQNRIDPDKLSSGYKDVILWFSFGASCFFILLAIMGLV  
FICVKNNGNMRCTICI
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Glycosylation origin

Human

Reporter Protein

Firefly luciferase

Storage

- 80 °C, avoid freeze/thaw cycles.

For more information

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Message object should contain:

“ Influenza H3 pseudovirus – #251215 - H3HK”.

Intended use

For Research Use Only. Not for Use in Diagnostic Procedures.
Not Meant for Resale.