IVANO BIOSCIENCE

Influenza H3 Pseudovirus

A/Hong Kong/1/1968 (H3N2) strain

Luciferase reporter

Lot #250217

Certificate of Analysis

March 03, 2025

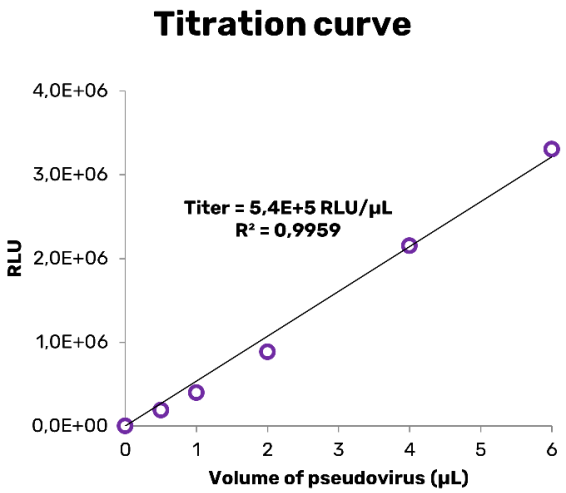
Version 2

1. Summary

This certificate is a functional validation for the lot #250217 of an avian HA pseudotyped pseudovirus, A/Hong Kong/1/1968 (H3N2) strain. The titer is 5.4 x 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 – 1.5 – 2 –4 – 6 µL/well
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)



Volume of pseudovirus (µL)	RLU 1	RLU 2	Mean RLU	CV RLU	Fold *vs* Background
0	1,0E+02	1,2E+02	1,1E+02	1,1E+01	1,0E+00
0,5	1,8E+05	2,0E+05	1,9E+05	1,2E+04	1,7E+03
1	4,1E+05	3,8E+05	4,0E+05	2,1E+04	3,5E+03
2	9,2E+05	8,4E+05	8,8E+05	5,7E+04	7,9E+03
4	2,1E+06	2,2E+06	2,2E+06	5,1E+04	1,9E+04
6	3,3E+06	3,3E+06	3,3E+06	3,4E+04	2,9E+04

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 (#250217) can transduce the target cells. The titer is 5.4 x 10^5 RLU/µL. Using 1 µL/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 #250217 could be used to perform approximately 1,000 reactions or 10 x 96-well plates, according to IVANO Bioscience's protocol (available upon request). Note that a volume of 8 µL of pseudovirus provided an overflow signal upon analysis

3. Neutralization assay

Target cells	HEK293T cells
Volume of pseudovirus	1 µL / 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)

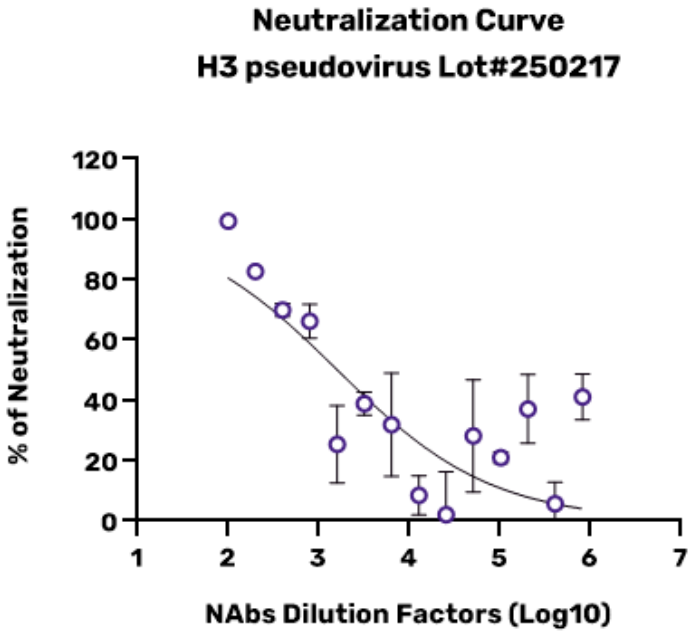


Figure 2: Neutralization curve
A starting dilution of 1/100 of a neutralizing antibody was serially diluted in a final volume of 50 µL of medium and incubated for 1 hour at 37 °C, with 1 µL of pseudovirus, in a 96-well plate. Then, 50 µL of medium, containing 10,000 cells, was seeded in each well and incubated for 72 hours. 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. 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 (#250217) 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) (GenBank: AAK51719.1)

Pseudotyping sequence

MNTQILVFALIAIIPANADKICLGHHAVSNGTKVNTLTERGVEVVNATETVERTNIP
RICSKGKRTVDLGQCGLLGTITGPPQCDQFLEFSADLIIERREGSDVCYPGKFVN
EEALRQILRESGGIDKEAMGFTYSGIRTNGATSACRRSGSSFYAEMKWLLSNTD
NAAFPQMTKSYKNTRKSPALIVWGIHHSVSTAEQTKLYGSGNKLVTVGSSNYQ
QSFVPSPGARPQVNGLSGRIDFHWLMLNPNDTVTFSFNGAFIAPDRASFLRGKS
MGIQSGVQVDANCEGDCHHSGGTIISNLPFQNIDSRAVGKCPRYVKQRSLLLAT
GMKNVPEIPKGRGLFGAIAGFIENGWEGLIDGWYGFRHQNAQGEGTAADYKST
QSAIDQITGKLNRLIEKTNQQFELIDNEFNEVEKQIGNVINWTRDSITEVWSYNAE
LLVAMENQHTIDLADSEMDKLYERVKRQLRENAEEDGTGCFEIFHKCDDDCMA
SIRNNTYDHSKYREEAMQNRIQIDPVKLSSGYKDVILWFSFGASCFILLAIVMGLV
FICVKNGNMRCTICI

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 – #250217".

Intended use

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