IVANO BIOSCIENCE

Influenza H5 Pseudovirus

A/VietNam/1194/2004 (H5N1) strain

Luciferase reporter

Lot #250217

Certificate of Analysis

July 11, 2025

Version 2

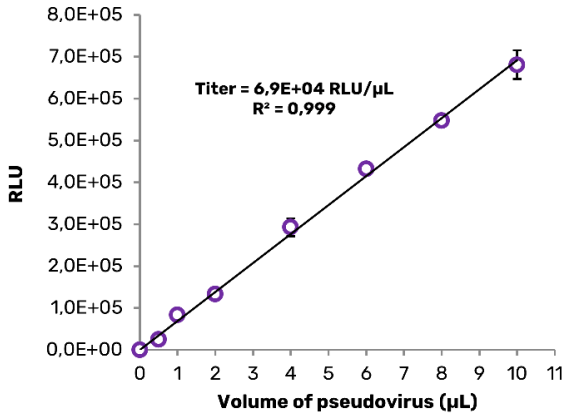
1. Summary

This certificate is a functional validation for the lot #250217 of an avian HA pseudotyped pseudovirus, A/VietNam/1194/2004 (H5N1) strain. The titer is 6.9 x 10^4 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 µL/well
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)

Titration curve



Volume of pseudovirus (µL)	RLU 1	RLU 2	Mean RLU	CV RLU	Fold *vs* Background
0	5.7E+01	4.2E+01	5.0E+01	1.1E+01	1.0E+00
0.5	1.8E+04	3.1E+04	2.5E+04	9.6E+03	5.0E+02
1	**8.5E+04**	**8.3E+04**	**8.4E+04**	**1.2E+03**	**1.7E+03**
2	1.3E+05	1.4E+05	1.3E+05	3.4E+03	2.7E+03
4	3.1E+05	2.8E+05	2.9E+05	2.1E+04	5.9E+03
6	4.4E+05	4.3E+05	4.3E+05	4.3E+03	8.7E+03
8	5.6E+05	5.4E+05	5.5E+05	1.3E+04	1.1E+04
10	7.1E+05	6.6E+05	6.8E+05	3.4E+04	1.4E+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 H5 pseudovirus (#250217) can transduce the target cells. The titer is 6.9 x 10^4 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).

3. Neutralization assay

Target cells	HEK293T cells
Volume of pseudovirus	1 µL/well
Neutralizing antibody (Nabs)	Anti-H5 surface glycoprotein - In-house antibody
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)

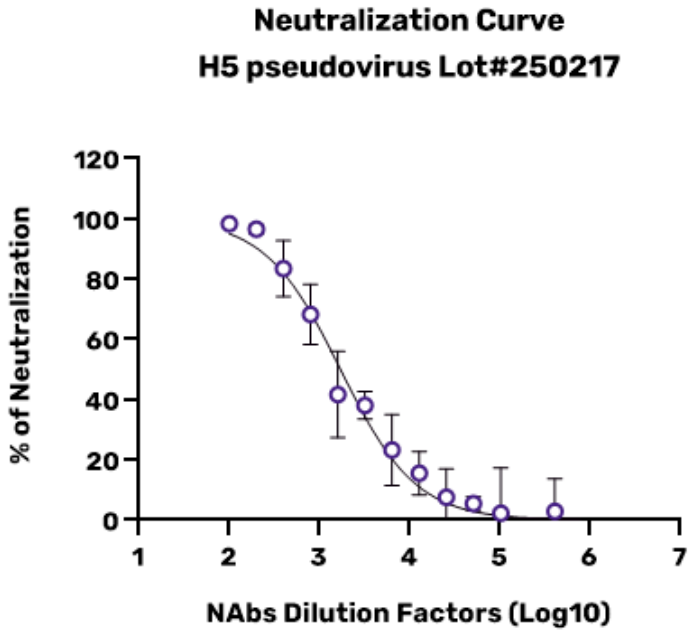


Figure 2: Neutralization curve
A starting dilution of 1/100 of a neutralizing antibody, was serially diluted in a final volume of 50 µL of medium and incubated for 1 hour at 37 °C, with 1 µL of pseudovirus, in a 96-well plate. Then, 50 µL of medium, containing 10,000 cells, was seeded in each well and 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 H5 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/VietNam/1194/2004 (H5N1) (GENBANK: EF541402.1)

Pseudotyping sequence

MEKIVLLFAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGK
LCDLDGVKPLILRDCSVAGWLLGNPMCDEFINVPEWSYIVEKANPVNDLCYPGD
FNDYEELKHLLSRINHFEKIQIIPKSSWSSHEASLGVSSACPYQGKSSFFRNVVW
LIKKNSTYPTIKRSYNNTNQEDLLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTL
NQRLVPRIATRSKVNGQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKG
DSTIMKSELEYGNCNTKCQTPMGAINSSMPFHNIHPLTIGECPKYVKSNRLVLAT
GLRNSPQRERRRKKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQGSGYAA
DKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDV
WTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKC
DNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGIYQILSIYSTVASSLALAI
MVAGLSLWMCSNGSLQCRICI

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 H5 pseudovirus – #250217".

Intended use

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