IVANO BIOSCIENCE

Avian Influenza H5 Pseudovirus

A/VietNam/1194/2004 (H5N1)

Luciferase reporter

Lot #240502

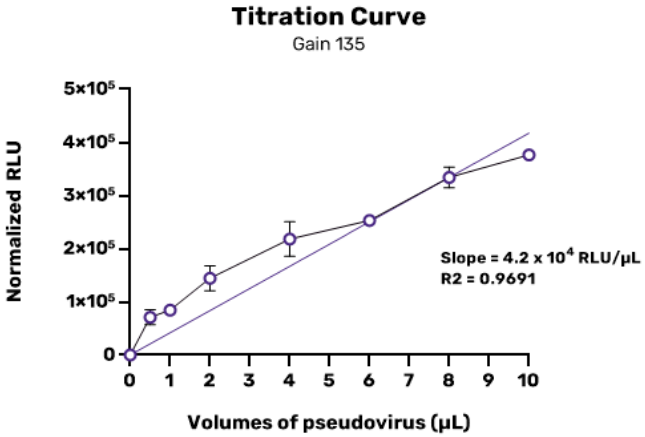
Certificate of Analysis

1. Summary

This certificate is a functional validation for the lot #240502 of an avian HA pseudotyped pseudovirus, strain A/VietNam/1194/2004 (H5N1). The titer is 4.2 x 10^4 RLU/µL. A volume of 1 mL of lot #240502 can be used to perform approximately 1,000 reactions or 10 x 96-well plates (according to the IVANO Bioscience protocol available upon request).

2. Transduction efficiency assay

Target cells	HEK293-T cells
Volume of pseudovirus	0 - 0.5 – 1 – 2 – 4 - 6 - 8 and 10 µL/well
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)



Volume of pseudovirus (µL)	Raw Ddata Mean	Fold vs Background
0	1,02E+02	Background
0,5	7,11E+04	6,97E+02
1	8,43E+04	8,27E+02
2	1,45E+05	1,42E+03
4	2,18E+05	2,14E+03
6	2,53E+05	2,48E+03
8	3,34E+05	3,28E+03
10	3,77E+05	3,69E+03

Figure 1: Transduction efficiency curve

A volume range of pseudovirus was mixed in a final volume of 50 µL of culture complete medium, in a 96-well plate. Then, an additional 50 µL of transduction medium containing 10 000 cells was seeded in each well. Luciferase expression analysis was performed 72 hours post-infection by a luminescence microplate reader.

Conclusion:
The H5 pseudovirus (#240502) can transduce the target cells. The titer is: 4.2 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 #240716 can be used to perform approximately 1,000 reactions or 10 x 96-well plates (according to the IVANO Bioscience protocol available upon request).

3. Neutralization assay

Target cells	HEK293-T cells
Volume of pseudovirus	1 µL/well
Neutralizing antibody (Nabs)	Anti-H5 surface glycoprotein - AB00798-10.0
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 – (Gain: 135)

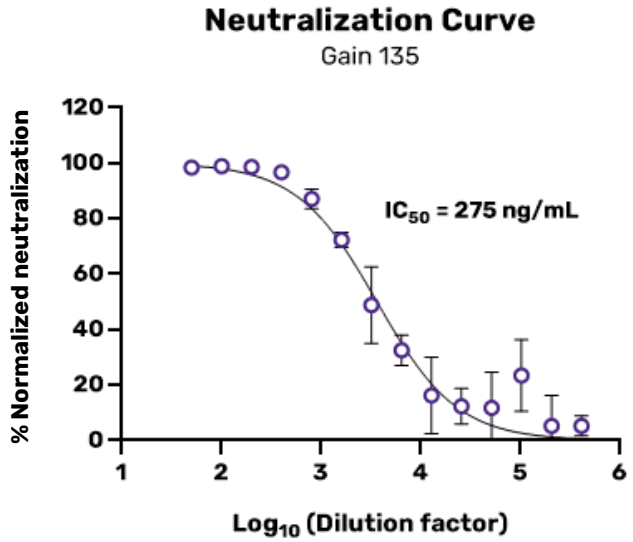


Figure 2: Neutralization curve

A monoclonal neutralizing antibody (AB00798-10.0) at 20 µg/mL was 2-fold serial diluted on fourteen dilutions in a final volume of 50 µL of transduction medium and incubated for 1 hour at 37 °C with 1 µL of pseudovirus, in a 96-well plate. Then, an additional 50 µL of transduction medium containing 10 000 HEK293-T cells was seeded in each well. One hundred µL of complete medium were added 24 hours after pseudovirus addition. The Luciferase expression was analyzed 72 hours post-transduction by removing 100 µL of medium followed by addition of 100 µL of Bright-Glo™ Luciferase buffer per well and a 2 minutes incubation. Data in relative unit luminescence (RLU) were obtained from the analysis of 150 µL of cell lysate in a white 96-well plate 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 H5 pseudovirus #240502 can be efficiently neutralized by neutralizing antibodies.

4. Additional information

Instruction of Uses	We recommend determining the titer in your lab's conditions before performing any experiments. Handle under biosafety level-2.
Pseudovirus	Replication incompetent. 3rd generation lentiviral vector, incompetent replication and non-toxic.
Pseudotyping	Influenza hemagglutinin, strain A/VietNam/1194/2004 (H5N1) (GENBANK: EF541402.1)
Glycosylation origin	Human
Reporter protein	Firefly luciferase
Storage	- 80 °C, avoid freeze/thaw cycles
Transduction medium	DMEM (without phenol red, High glucose, glutamine and sodium pyruvate) + 2% FBS + 25 mM Hepes + 1X glutamax
Complete medium	DMEM (without phenol red, High glucose, glutamine and sodium pyruvate) + 10% FBS + 25 mM Hepes + 1X glutamax
For more information	mathias.mangion@ivanobioscience.com Message object should contain: "H5 - lot #240502"