IVANO BIOSCIENCE

Influenza H7 Pseudovirus

A/Shanghai/4664T/2013 (H7N9)

Luciferase reporter

Lot #240716

Certificate of Analysis

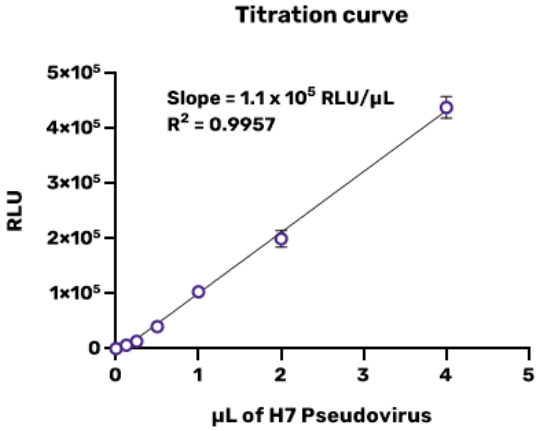
Version 2

1. Summary

This certificate is a functional validation for the lot #240716 of an avian HA pseudotyped pseudovirus, strain A/Shanghai/4664T/2013 (H7N9). The titer is 1.1 x 10^5 RLU/µL. A volume of 1 mL of lot #240716 can be used to perform approximately 1,000 reactions or 10 x 96-well plates.

2. Transduction efficiency assay

Target cells	HEK293-T cells
Volume of pseudovirus	0 – 0.125 – 0.25 – 0.5 – 1 – 2 – 4 µL/well
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)



Volumes of pseudovirus (µL)	Raw Data Mean	Fold vs Background
0	1,75E+01	Background
0.125	6,36E+03	3,64E+02
0,25	1,37E+04	7,82E+02
0,5	3,99E+04	2,28E+03
1	1,03E+05	5,91E+03
2	2,00E+05	1,14E+04
4	4,38E+05	2,50E+04

Figure 1: Transduction efficiency curve
A volume range of pseudoviruses was mixed in a final volume of 50 µL of culture complete medium, in a 96-well plate. Then, 50 µL containing 10,000 cells was seeded in each well. Luciferase expression was detected 72 hours post-transduction by adding a luciferase reagent (Bright Glo, Promega), using a white 96-well plate. Data are expressed in relative unit luminescence (RLU).

Conclusion:
The pseudovirus (#240716) can transduce the target cells. The titer is: 1.1 x 10^5 RLU/µL. Using 1 µL/reaction of pseudovirus in a 96-well plate can 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-H7N9 Hemagglutinin / Rabbit MAb, NAB-FLU-001
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)

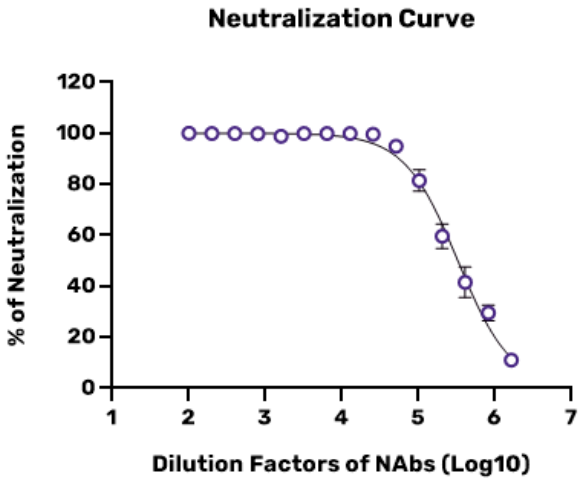


Figure 2: Neutralization curve

A monoclonal neutralizing antibody (IVANO Bioscience, NAB-FLU-001), at a starting dilution of 1/100, was serial diluted in a final volume of 50 µL of complete medium and incubated for 1 hour at 37 °C, with 1 µL of pseudovirus, in a 96-well plate. Then, an additional 50 µL containing 10 000 cells was seeded in each well and incubated for 72 hours. Finally, an additional 100 µL of Bright-Glo™ Luciferase buffer 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 with 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:
This H7 pseudovirus (#240716) 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/Shanghai/4664T/2013 (H7N9) (GENBANK: KC853228.1).
Glycosylation origin	Human.
Reporter protein	Firefly luciferase.
Storage	- 80 °C, avoid freeze/thaw cycles.
For more information	mathias.mangion@ivanobioscience.com Message object should contain: "H7 - #240716".
Intended use	For Research Use Only. Not for Use in Diagnostic Procedures. Not Meant for Resale.

5. Annexe

Neutralization data

Dilution factors (Log10)	Mean	CV
2.0	40.5	6.4
2.3	44.0	5.7
2.6	50.0	0.0
2.9	156.5	139.3
3.2	853.0	1091.8
3.5	60.0	32.5
3.8	68.0	31.1
4.1	85.5	7.8
4.4	328.0	384.7
4.7	3763.5	1571.9
5.0	13724.5	3099.2
5.3	29793.5	3578.7
5.6	43183.0	4457.6
5.9	52025.5	2296.0
6.2	65681.5	1414.9
No NAbs	73840.5	3683.3
Cell Only	72.8	56.0