

**Influenza H5 Pseudovirus  
A/VietNam/1194/2004 (H5N1) Strain**

**Luciferase reporter**

**Lot #260216 – H5VN**



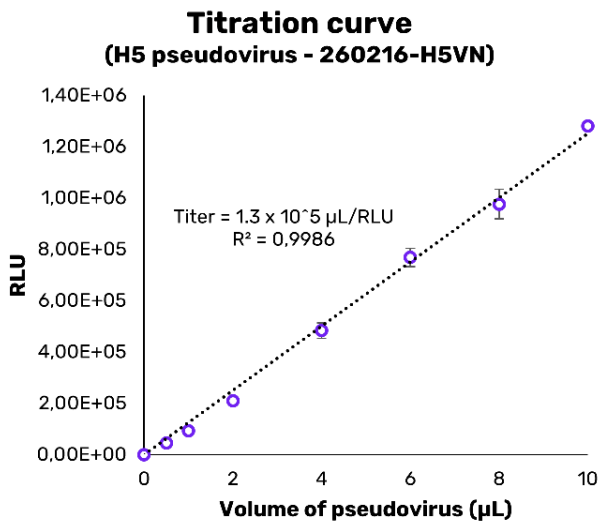
**Certificate of Analysis**

# 1. Summary

This certificate provides a functional validation of the Influenza H5 pseudovirus, A/VietNam/1194/2004 (H5N1) strain, lot #260216 – H5VN. The titer is  $1.3 \times 10^5$  RLU/ $\mu$ L. A 1,000-fold-to-background ratio is obtained with 1  $\mu$ L per well (96-well plate). Hence, a volume of 1 mL is sufficient to perform approximately 1,000 reactions, or  $10 \times 96$ -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  $\mu$ L/well  
**Detection signal** Luminescence (firefly luciferase)  
**Detection method** Microplate reader Biotek Synergy H1 (Gain: 135)



Volume of pseudovirus ( $\mu$ L)	RLU 1	RLU 2	MEAN RLU	CV RLU	Fold vs background
0	1,5E+02	7,5E+01	1,1E+02	5,0E+01	1,0E+00
0,5	4,7E+04	4,5E+04	4,6E+04	1,4E+03	4,1E+02
<b>1</b>	<b>1,0E+05</b>	<b>8,1E+04</b>	<b>9,3E+04</b>	<b>1,6E+04</b>	<b>8,4E+02</b>
2	2,1E+05	2,1E+05	2,1E+05	2,7E+03	1,9E+03
4	4,6E+05	5,1E+05	4,8E+05	3,1E+04	4,4E+03
6	7,4E+05	7,9E+05	7,7E+05	3,5E+04	7,0E+03
8	9,4E+05	1,0E+06	9,8E+05	5,8E+04	8,8E+03
10	1,3E+06	1,3E+06	1,3E+06	7,4E+03	1,2E+04

**Figure 1: Transduction efficiency curve**

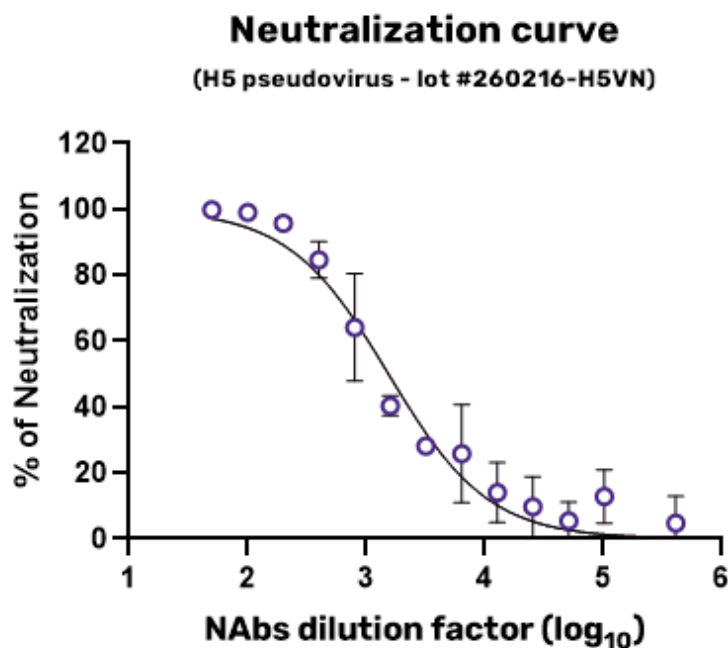
A volume range of pseudovirus was mixed in a final volume of 50  $\mu$ L of medium, in a 96-well plate. Then, 50  $\mu$ L of medium containing 10,000 cells was seeded in each well. On the day of analysis, an additional 100  $\mu$ L of Bright-Glo Luciferase reagent was added in each well and incubated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

**Conclusion**

The Influenza H5 pseudovirus, A/VietNam/1194/2004 (H5N1) strain (lot #260216 – H5VN), is capable of transducing target cells. The titer is  $1.3 \times 10^5$  RLU/ $\mu$ L. Using 1 microliters of pseudovirus per reaction in a 96-well plate results in a 1,000-fold increase in RLU compared to the background. Accordingly, 1 mL of lot #260216 – H5VN can be used to perform approximately 1,000 reactions, or  $10 \times 96$ -well plates, according to IVANO Bioscience’s protocol (available upon request)

### 3. Neutralization assay

<b>Target cells</b>	HEK293T cells
<b>Volume of pseudovirus</b>	1 $\mu$ L/well
<b>Neutralizing antibody (Nabs)</b>	Anti-H5 surface glycoprotein [Ab00798-10.0]
<b>Detection signal</b>	Luminescence (firefly luciferase)
<b>Detection method</b>	Microplate reader Biotek Synergy H1 (Gain: 135)



**Figure 2: Neutralization curve**

A monoclonal neutralizing antibody ([Ab00798-10.0]), at a starting dilution of 1/50, was serially diluted in a final volume of 50  $\mu$ L of complete medium and incubated for 1 hour at 37 °C, with 1  $\mu$ L of pseudovirus, in a 96-well plate. Then, an additional 50  $\mu$ L containing 10 000 cells was seeded in each well and incubated for 72 hours. Finally, an additional 100  $\mu$ L 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  $\mu$ L 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 in 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, A/VietNam/1194/2004 (H5N1) strain (lot #260216 – H5VN) 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

3<sup>rd</sup> generation lentiviral vector, incompetent replication and non-toxic

### Pseudotyping

Influenza hemagglutinin of the Influenza H5 pseudovirus, A/Viet-Nam/1194/2004 (H5N1) strain.(Genbank EF541402.1)

### Pseudotyping sequence

```
MEKIVLLFAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGK  
LCDLDGVKPLILRDCSVAGWLLGNPMCDEFINVPEWSYIVEKANPVNDLCYPGD  
FNDYEELKHLLSRINHFEKIQIIPKSSWSSHEASLGVSSACPYQGKSSFFRNWWW  
LIKKNSTYPTIKRSYNNTNQEDLLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTL  
NQRLVPRIATRSKVNGQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKG  
DSTIMKSELEYGNCNTKCQTPMGAINSSMPFHNIHPLTIGECPKYVKSRLVLT  
GLRNSPQRERRRKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQGSYAA  
DKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDV  
WTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKC  
DNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGIYQILSIYSTVASSLALAI  
MVAGLSLWMCSNGSLQCRICI
```

### Glycosylation origin

Human

### Reporter Protein

Firefly luciferase

### Storage

- 80 °C, avoid freeze/thaw cycles

### For more information

[mathias.mangion@ivanobioscience.com](mailto:mathias.mangion@ivanobioscience.com)

Message object should contain: "H5 pseudovirus – lot #260216 – H5VN "

### Intended use

For Research Use Only

Not for Use in Diagnostic Procedures, not Meant for Resale