IVANO BIOSCIENCE

Pseudovirus H5 (luciferase)
A/Viet Nam/1194/2004 (H5N1)

Lot #PsVL-H5VN9404-231223

Quality control report

1. Summary

The lot number #PsVL-H5VN9404-231223 is a lentivirus-based pseudovirus pseudotyped with the HA protein of the A/Viet Nam/1194/2004 (H5N1) variant. This quality control report demonstrates that the lot #PsVL-H5VN9404-231223 is efficient for cell transduction and can be effectively neutralized by a standard neutralizing antibody.

2. Transduction efficiency assay

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

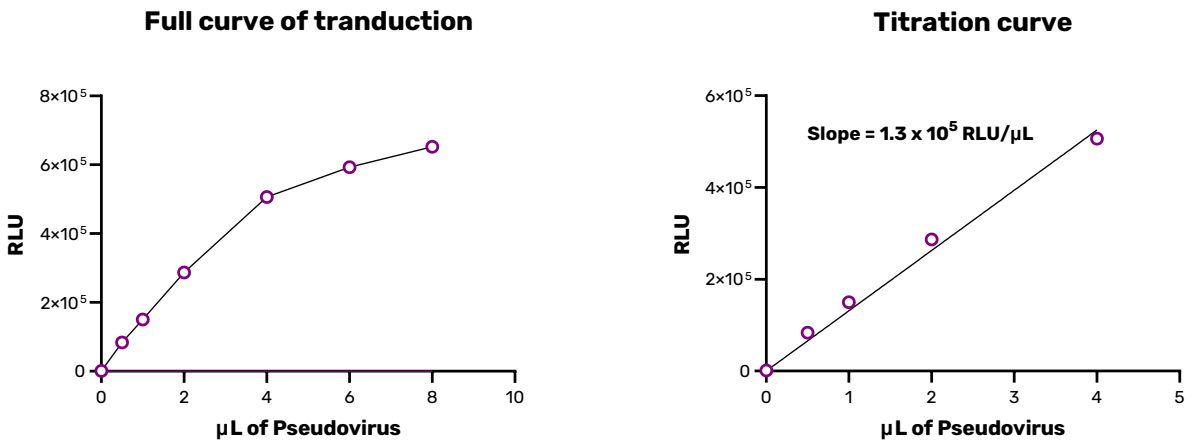

Figure 1: Transduction efficiency curve

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 containing 20 000 HEK293-T cells was seeded in each well. Luc expression analysis was performed 72 hours post-infection by a luminescence microplate reader.

Conclusion	The H5 pseudovirus (#PsVL-H5VN9404-231223) can transduce the target cells. This batch titer is : 1.3x10^5 RLU/µL.

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

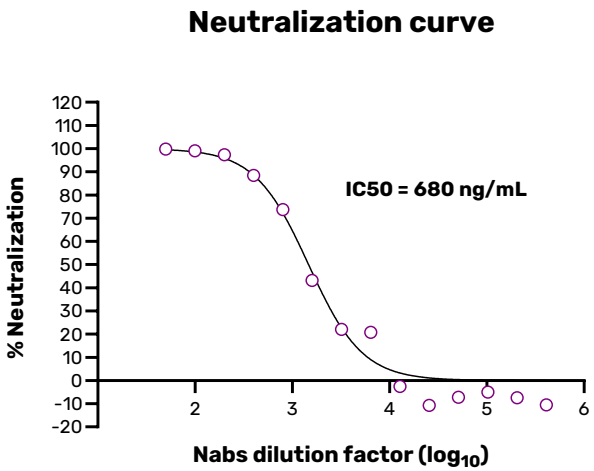

Figure 2: Neutralization curve

A monoclonal neutralizing antibody (AB00798-10.0) at 1 mg/mL 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 20 000 HEK293-T 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	The H5 pseudovirus #PsVL-H5VN9404-231223 can be efficiently neutralized by neutralizing antibodies.

3. Additional information

Caution	We recommend determining the optimal pseudovirus volume to use according to your specific experimental conditions
Pseudovirus	Replication incompetent. Handing in a BSL-2 laboratory
Pseudotyping	Influenza hemagglutinin H5 from the 2004 outbreak in Viet Nam (GENBANK: EF541402.1)
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: "#PsVL-H5VN9404-231223 "