

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

Lot #PsVL-H5VN9404-231223



Quality control report

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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.

Transduction efficiency assay

HEK293-T cells Target cells

Volume of pseudovirus 0 - 0.5 - 1 - 2 - 4 - 6 and $8 \mu L/well$

Detection signal Luminescence (firefly luciferase)

Detection method Microplate reader

Full curve of tranduction

8×105 6×105 **2** 4×10⁵ 2×10⁵ 8 10 µL of Pseudovirus

Titration curve

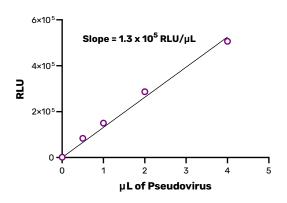


Figure 1: Transduction efficiency curve

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: 1.3x10⁵ RLU/µL.

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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

Neutralization curve

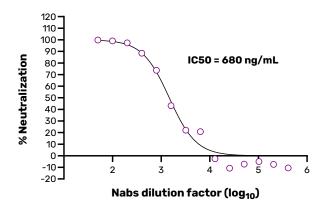


Figure 2: Neutralization curve

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.

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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 <u>mathias.mangion@ivanobioscience.com</u>

Message object should contain: "#PsVL-H5VN9404-

231223"