



Ebola Pseudovirus Sudan Variant

Luciferase reporter

Lot #251215-EBS



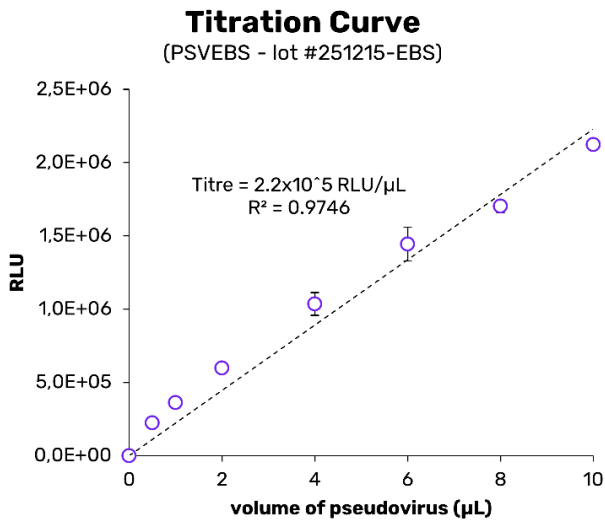
Certificate of Analysis

1. Summary

This certificate provides a functional validation of the Ebola pseudovirus, Sudan, lot #251215-EBS. The titer is 2.2×10^5 RLU/ μ L. A 1,000-fold-to-background ratio is obtained with 1 μ L per well (96-well plate). Hence, a volume of 1 mL is sufficient to perform approximately 1,000 reactions, or 10×96 -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 μ L/well
Detection signal Luminescence (firefly luciferase)
Detection method Microplate reader Biotek Synergy H1 (Gain: 135)



Volume of pseudovirus (μ L)	RLU 1	RLU 2	Mean	CV	Fold to Background
0	2,2E+02	2,1E+02	2,1E+02	5,7E+00	1,0E+00
0,5	2,1E+05	2,4E+05	2,2E+05	2,3E+04	1,0E+03
1	3,3E+05	3,9E+05	3,6E+05	4,0E+04	1,7E+03
2	5,9E+05	6,0E+05	6,0E+05	5,9E+03	2,8E+03
4	9,8E+05	1,1E+06	1,0E+06	7,7E+04	4,9E+03
6	1,4E+06	1,5E+06	1,4E+06	1,2E+05	6,8E+03
8	1,7E+06	1,7E+06	1,7E+06	4,3E+04	8,0E+03
10	2,1E+06	2,1E+06	2,1E+06	2,3E+04	1,0E+04

Figure 1: Transduction efficiency curve

A volume range of pseudovirus was mixed in a final volume of 50 μ L of medium, in a 96-well plate. Then, 50 μ L of medium containing 10,000 cells was seeded in each well. On the day of analysis, an additional 100 μ 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 μ L of the cell lysate, using a microplate reader. Data are expressed in relative unit luminescence (RLU).

Conclusion

The Ebola pseudovirus, Sudan variant (lot #251215-EBS), is capable of transducing target cells. The titer is 2.2×10^5 RLU/ μ 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 #251215-EBS can be used to perform approximately 1,000 reactions, or 10×96 -well plates, according to IVANO Bioscience’s protocol (available upon request)

3. Neutralization assay

Target cells	HEK293T cells
Volume of pseudovirus	1 μ L/well
Neutralizing antibody (Nabs)	Anti-Ebola surface glycoprotein [KZ52] , Ab00690-10.0
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)

Neutralization curve

(PSVEBS - Lot #251215-EBS)

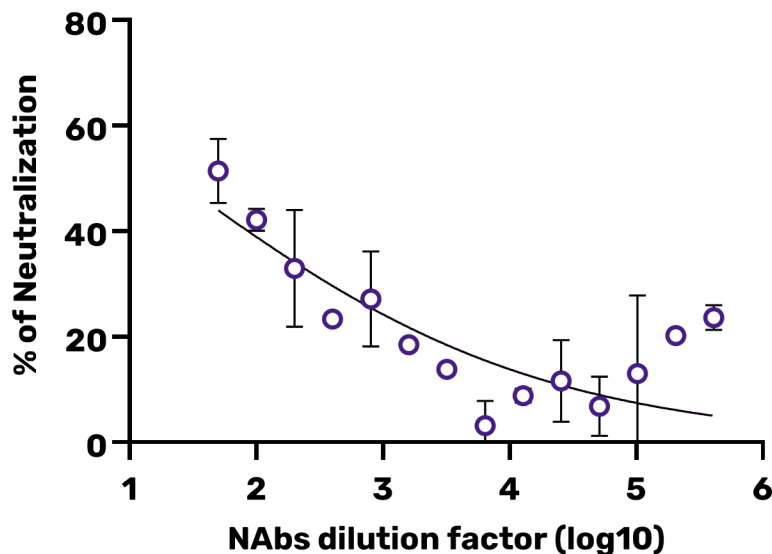


Figure 2: Neutralization curve

A monoclonal neutralizing antibody (Ab00690-10.0), at a starting dilution of 1/50, was serially diluted in a final volume of 50 μ L of complete medium and incubated for 1 hour at 37 °C, with 1 μ L of pseudovirus, in a 96-well plate. Then, an additional 50 μ L containing 10,000 cells was seeded in each well and incubated for 72 hours. Finally, an additional 100 μ 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 μ 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 into wells as 100% neutralization and cells transduced by pseudoviruses without any NAbs as 0% neutralization. Data are representative of duplicates.).

Conclusion

The Ebola pseudovirus, Sudan variant (lot #251215-EBS) 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 rd generation lentiviral vector, incompetent replication and non-toxic
Pseudotyping	Glycoprotein of the Ebola pseudovirus, Sudan variant Genbank: WEY07040.1
Pseudotyping sequence	<pre>MGSLSLQLPRDKFRKSSFFVWVILFQKAFSMPLGVVTNSTLEVTEIDQLVCKD HLASTDQLKSVGLNLEGSGVSTDIPSATKRWGFRSGVPPKVVSYEAGEWAENC YNLEIKKPDGSECLPPPPDGVRGFPCRYVHKAQGTGPCPGDYAFHKDGAFFL YDRLASTVIYRGVNFAEGVIAFLILAKPKETFLQSPPIREAVNYTENTSSYYATSYL EYEIENFGAQHSTTLFKIDNNTFVRLDRPHTPQFLFQLNDTIHLHQQLSNTTGRLI WTLNANINADIGEWAFWENKKNLSEQLRGEELSFEALSNETEDDDAASSRITK GRISDRATRKYSIDLVPKNSPGMVPLHIPEGETTLPSQNSTEGRRVGVNTQETIT ETAATIIGTNGNHMQISTIGIRPSSNQIPSSSPTTAPSPEAQPTTHTSGPSVMAT EETTPPGSSPGPTTEAPTLLTPENITTAVKTVLPQUESTSNGLITSTVTGILGSLG LRKRSRRQTNTKATGKCNPNLHYWTAQEQHNAAGIAWIPYFGPGAEGIYTEGL MHNQNALVCGLRQLANETTQALQLFLRATTELRTYITILNRKAIDFLLRRWGGTC RILGPDCCEPHDWTKNITDKINQIIHDFIDNPLPNQDNDNWWTGWRQWIPAGI GITGIIAIIALLCVCKLLC</pre>
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: "PSVEBS – lot #251215-EBS "
Intended use	For Research Use Only Not for Use in Diagnostic Procedures, not Meant for Resale