



SARS-CoV-2 Pseudovirus Alpha (B.1.1.7) Variant

Luciferase reporter

Lot #251201



Certificate of Analysis

1. Summary

This certificate provides a functional validation of the SARS-CoV-2 pseudovirus, Alpha (B.1.1.7), lot #251201. The titer is 5.4×10^5 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	HEK293 cells (ACE2 ⁺)
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)

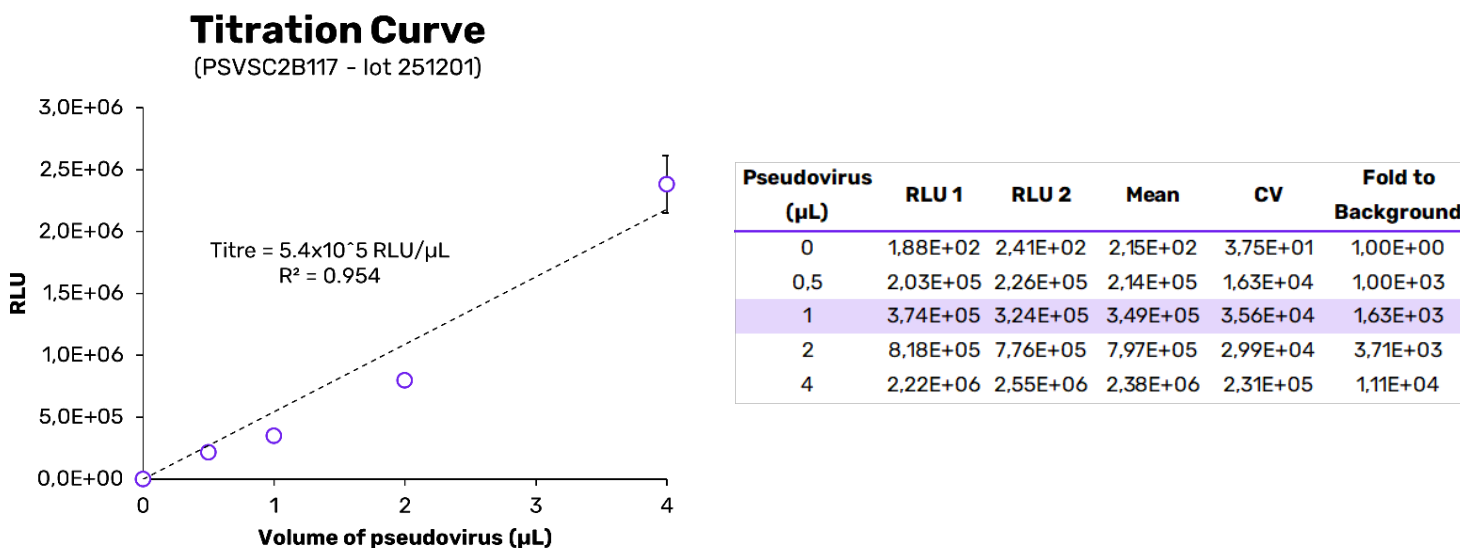


Figure 1: Transduction efficiency curve

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 SARS-CoV-2 pseudovirus, Alpha (B.1.1.7) variant (lot #251201), is capable of transducing target cells. The titer is 5.4×10^5 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 #251201 can be used to perform approximately 1,000 reactions, or 10×96 -well plates, according to IVANO Bioscience's protocol (available upon request)

Note that a volume of 6 μ L of pseudovirus provided an overflow signal upon analysis .

3. Neutralization assay

Target cells	HEK293 cells (ACE2 ⁺)
Volume of pseudovirus	1 μ L/well
Neutralizing antibody (Nabs)	Anti-Spike Protein (RBD) [CV30] , Ab02019-12.1
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)

Neutralization curve

(PSVSC2B117 - Lot #251201)

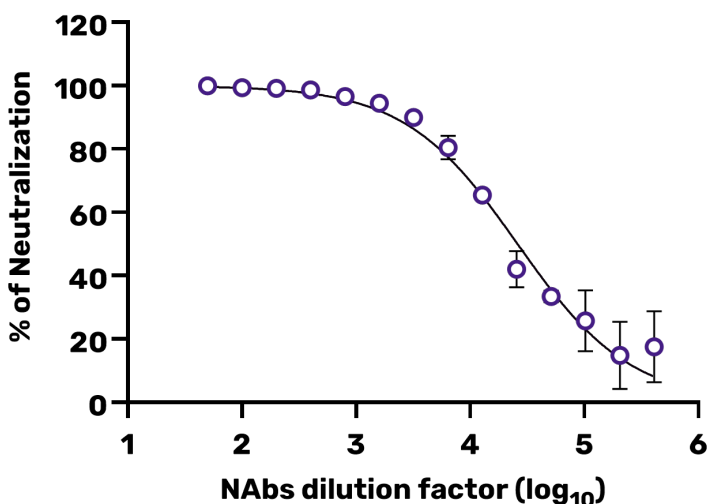


Figure 2: Neutralization curve

A monoclonal neutralizing antibody (Ab02019-12.1), 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 in wells as 100% neutralization and cells transduced by pseudoviruses without any NAbs as 0% neutralization. Data are representative of duplicates.).

Conclusion

The SARS-CoV-2 pseudovirus, Alpha (B.1.1.7) variant (lot #251201) 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	Spike glycoprotein of the SARS-CoV-2 pseudovirus, Alpha (B.1.1.7) variant GISAID Accession Number: EPI_ISL_1164753
AA Substitutions	Spike A570D, Spike D614G, Spike D1118H, Spike H69del, Spike N501Y, Spike P681H, Spike S982A, Spike T716I, Spike V70del, Spike Y144del
Pseudotyping sequence	MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVVYYPDKVFRSSVLHSTQDL FLPFFSNVTWFHAIISGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLD SKTQSLIVNATNVVIVKVEFCQFCNDPFLGVYHKNNKSWMESEFRVYSSANNC TFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFS ALEPLVDLPIGINITRFQTLALHRSYLTGPDSSSGWTAGAAAYVGYLQPRFTLL KYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNIT NLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPSTKL NDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNL DSKVGGNYNLYRLFRKSNLKPFRDISTEIQAGSTPCNGVEGFNCYFPLQSY GFQPTYGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTG TGVLTESNKKFLPFQQFGRDIDDDTTDAVRDPQTLEILDITPCSFGGVSVITPGTNT SNQVAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN NSYECDIPIGAGICASYQTQTNSHSRASSVASQSIIAYTMSLGAENSVAYSNNNSIA IPINFTISVTTEILPVSMTKTSVDCTMYICGDSSTECNLLLQYGSFCTQLNRALTG IAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSPKPSKRSFIEDLLFNK VTLADAGFIKQYGDCLGDIARDLCAQKFNGLTVLPLLTDEMIAQYTSALLAGT ITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQD SLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILARLDKVEAEV QIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKG YHLMSFPQSAPHGVVFLHVTVYVPAQEKNTTAPAICHGDKAHFPREGVVFVSNGT HWFVTQRNFYEPQIITHTHTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKY FKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK WPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCCLKGCCSCGSCC
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: "PSVSC2B117– lot #251201"
Intended use	For Research Use Only Not for Use in Diagnostic Procedures, not Meant for Resale