



SARS-CoV-2 Pseudovirus

D614G Variant

Luciferase reporter

Lot #250804



Certificate of Analysis

1. Summary

This certificate provides a functional validation of the SARS-CoV-2 pseudovirus, BA.1 variant, lot #250804. The titer is 1.2×10^6 RLU/ μ L. A fold-to-background ratio is obtained with 1 μ L per well (96-well plate). One milliliter 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	HEK293 cells (ACE2 ⁺)
Volume of pseudovirus	0 – 0.5 – 1 – 2 μ L/well (signal saturation occurs at ≥ 4 μ L)
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)

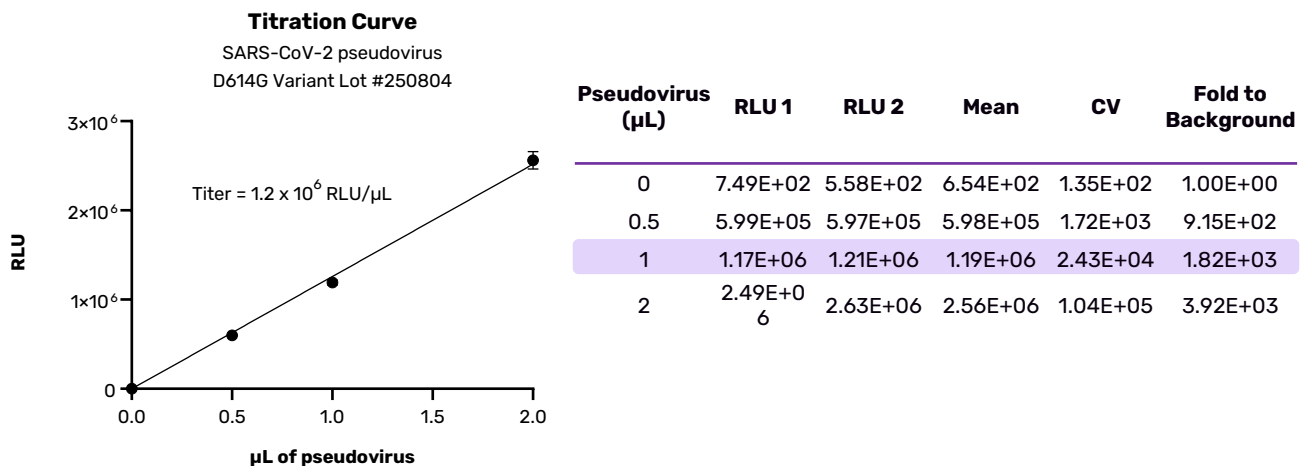


Figure 1: Transduction efficiency curve

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, D614G variant (lot #250804), is capable of transducing target cells. The titer is 1.2×10^6 RLU/ μ L. Using one 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 #250804 can be used to perform approximately 1,000 reactions, or 10 \times 96-well plates, according to IVANO Bioscience's protocol (available upon request). Note that signal saturation occurs at ≥ 4 μ L per well of pseudovirus.

3. Neutralization assay

Target cells	HEK293 cells (ACE2 ⁺)
Volume of pseudovirus	1 µL/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

SARS-CoV-2 pseudovirus
D614G Variant Lot #250804

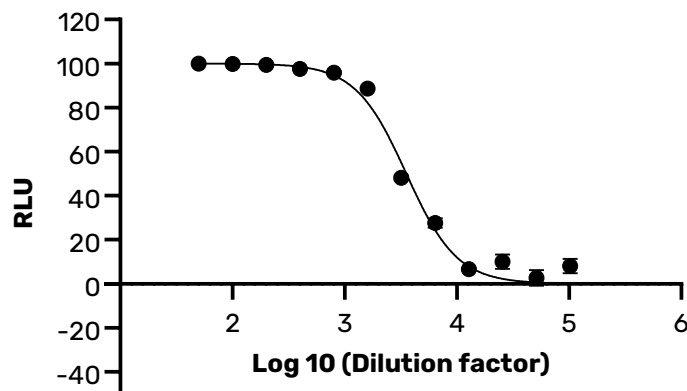


Figure 2: Neutralization curve

A monoclonal neutralizing antibody ([Ab02019-12.1](#)), at a starting dilution of 20 µg/mL, was serially diluted in a final volume of 50 µL of complete medium and incubated for 1 hour at 37 °C, with 2 µL of pseudovirus, in a 96-well plate. Then, an additional 50 µL containing 10 000 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 Omicron D614G variant SARS-CoV-2 pseudovirus (#250804) can be efficiently neutralized by neutralizing antibodies.

4. Additional information

Instruction 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 D614 variant SARS-CoV-2
Pseudotyping sequence	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVVYPDKVFRSSVLHSTQDLFLPFFS NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTSEKSNIRGWIFGTTLDLSTQSLIVN NATNVVIKVFCEFCNDPFLGVVYHKNKSWMESEFRVYSSANNCTFEYVVSQPFLMDL EGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPPQGFSALEPLVDLPIGINITRFQTL ALHRSYLTPGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKC TLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCV ADYSVLVNSASFSTFKCYGVSPTKLNLDLCTNVYADSFVIRGDEVRIAPGQTGKIADYN YKLPDDFTGCVIAWNSNNLDSKVGGNVNYLYRFRKSNLKPFERDISTEIQAGSTPCNG VEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNF NFNGLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLILDITPCSFGGVSVITPGT NTSNQVAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYE CDIPIGAGICASYQTQTNSPSRASSVASQSIAYTMSLGAENSVAYSNNNSIAIPTNFTISVTT EILPVSMTKTSVDCTMYICGDSTECNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQ VKQIYKTPPIKDFGGFNFSQILPDPSPKSRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAA RDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRF NGIGVTQNVLYENQKLIANQFNSAIGKIQDLSSTASALGKLQDVVNQNAQALNTLVKQLS SNFGAIVSSVLDILSRDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKM SECVLGQSKRVDFCGKGYHLSMFPQSAPHGVVFLHVTVVPAQEKNFHTTAPAICHGKA HFPREGVFSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDS FKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQ YIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCCLGKCCSCGSCC
Reporter protein	Firefly luciferase.
Storage	- 80 °C, avoid freeze/thaw cycles.
For more information	mathias.mangion@ivanobioscience.com Message object should contain: " D614G variant SARS-CoV-2 pseudovirus - #250804".
Intended use	For Research Use Only. Not for Use in Diagnostic Procedures. Not Meant for Resale.