



SARS-CoV-2 Pseudovirus

Omicron BA.1 variant

Luciferase reporter

Lot #241129



Certificate of Analysis

1. Summary

This certificate is a functional validation for the lot #241129 of the Omicron BA.1 variant SARS-CoV-2 pseudovirus. The titer is 2×10^4 RLU/ μ L. A volume of 1 mL can be used to perform around 500 reactions or 5 x 96-well plates, according to the IVANO Bioscience protocol available upon request.

2. Transduction efficiency assay

Target cells	HEK293 cells (ACE2 ⁺ , TMPRSS2 ⁺)
Volume of pseudovirus	0 - 1 - 2 - 4 - 6 - 8 - 10 μ L/well
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)

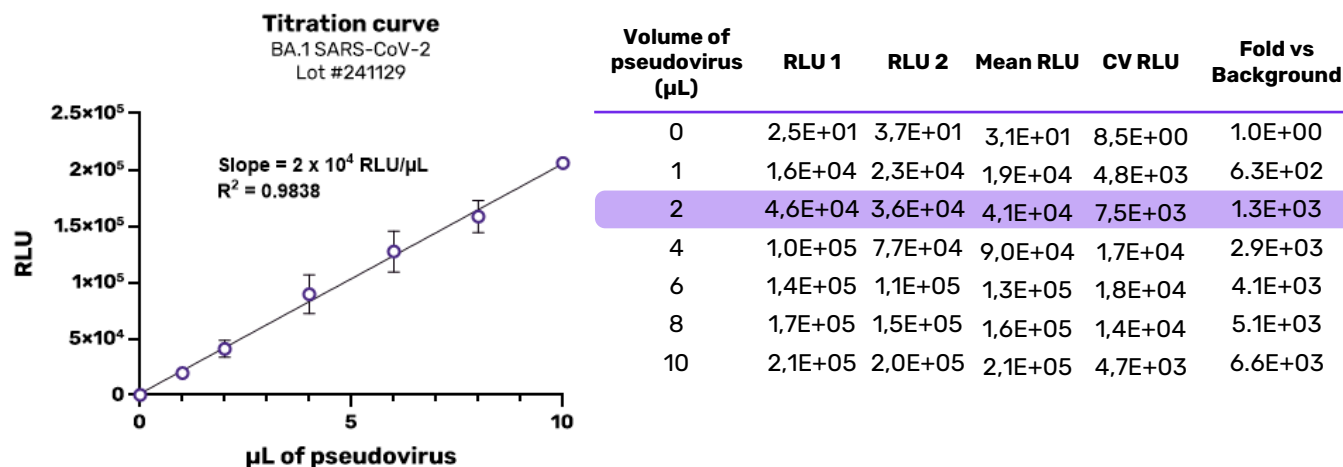


Figure 1: Transduction efficiency curve

A volume range of pseudovirus was mixed in a final volume of 50 μ L of transduction medium, in a 96-well plate. Then, 50 μ L containing 10 000 cells was seeded in each well. Luciferase expression was detected 72 hours post-transduction by adding a luciferase reagent (Bright Glo, Promega), using a white 96-well plate. Data are expressed in relative unit luminescence (RLU).

Conclusion:

The Omicron BA.1 variant SARS-CoV-2 pseudovirus (#241129) can transduce the target cells. The titer is 2×10^4 RLU/ μ L. Using 2 μ L/reaction of pseudovirus in a 96-well plate will yield a 1,000-fold increase in RLU compared to background. Therefore, 1 mL of lot #241129 could be used to perform approximately 500 reactions or 5 x 96-well plates, according to the IVANO Bioscience protocol available upon request.

3. Neutralization assay

Target cells	HEK293 cells (ACE2 ⁺ , TMPRSS2 ⁺)
Volume of pseudovirus	2 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)

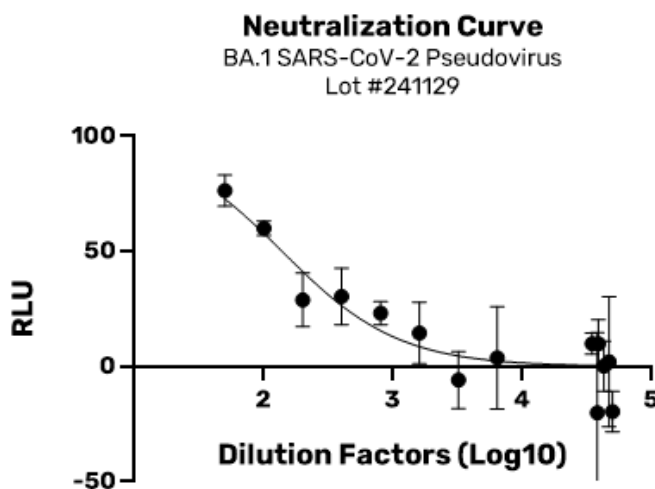


Figure 2: Neutralization curve

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 BA.1 variant SARS-CoV-2 pseudovirus (#241129) can be efficiently neutralized by neutralizing antibodies.

4. Freeze / thaw stability assessment

Target cells	HEK293 cells (ACE2 ⁺ , TMPRSS2 ⁺)
Volume of pseudovirus	2 µL/well
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)

Freeze-Thaw Cycle Stability Study

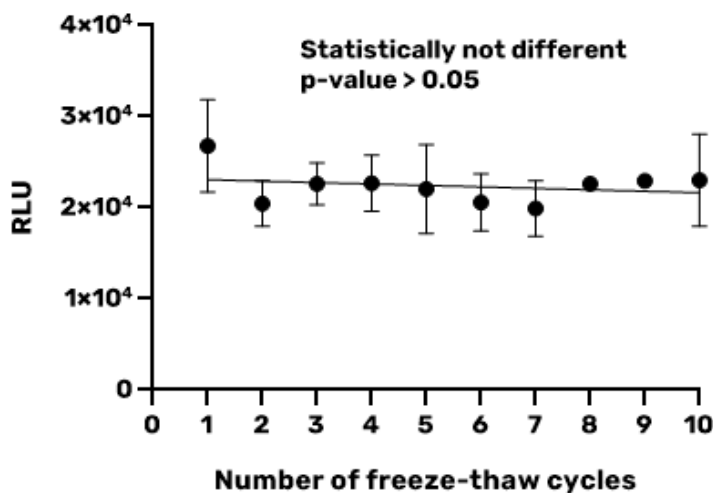


Figure 1: Freeze/thaw cycle transduction efficiency

This analysis was carried out using 10 vials of the same production batch where each vial was frozen/thawed between one and ten times. Then, a volume range of pseudovirus was mixed in a final volume of 50 µL of transduction medium, in a 96-well plate. Then, 50 µL containing 10 000 cells was seeded in each well. Luciferase expression was detected 72 hours post-transduction by adding a luciferase reagent (Bright Glo, Promega), using a white 96-well plate. Data are expressed in relative unit luminescence (RLU). The probability of significance between multiple groups was determined using analysis of variance. p-value < 0.05 indicates a statistically significant difference. p-value > 0.05 indicates a statistically not significant difference.

Conclusion:

Statistical analysis provided a p-value > 0.05 (P-value = 0.74). Hence, there is no significant difference in transduction efficiency between all tested conditions. Therefore, the batch is stable for at least 10 freeze/thaw cycles.

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 Omicron BA.1 variant SARS-CoV-2. The spike protein has an 18-aa cytoplasmic tail truncation for optimal infection. See sequence below: MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHVI SGTNGTKRFDNPVLPFNDGVYFASIEKSNIRGWIFGTTLDSKTQSLLVNNAATNVVIVKCEFCQCNDFP LDHKNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFNIDGYFKIYSKHTPII VRDLPGGFSALEPLVDLPIGINITRFQTLALHRSYLTGPDSSSGWTAGAAAYVGYLQPRTFLLKYN ENGTITDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFDEVFNATRFAS VYAWNRKRISNCVADYSVLYNLAPFFTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRIAPGQTG NIADYNYKLPDDFTGCVIAWNSNKLDSKVSNGNYLYRFRKSNLKPFRDISTEIQAGNKPCNGVA GFNCYFPLRSYSFRPTYGVGHQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNENGLKGTG VLTESNKKFLPFQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSIVTPTNTSNQVAVLYQGVNCTE VPVAIHADQLTPTWRVYSTGNSVNFQTRAGCLIGAEVNNNSYECDIPIGAGICASYQTQTKSHSRASSV ASQSIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYG SFCTQLKRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKYFGGFNFSQILPDPSPKPSKRSFIEDLLFNKVT LADAGFIKQYGDCLGDI AARDLICAQKFNGLTVLPPLLTDEMIQAQYTSALLAGTITSGWTFGAGAALQI PFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDLSSTASALGKLQDVVNHNAQALNTLVK QLSSKFGAISSVLNDFSRDLKVEAEVQIDRLITGRLQSLQTYVTQLIRAAEIRASANLAATKMSECVL GQSKRVDFCGKGYHLSFQPSAPHGVVFLHVTYVPAQEKNFPTAPAICHGDKAHFPREGVFVSNGT HWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGD SGINASVVNIQEKIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSC CSCLKGCCSCGSCC
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: " Omicron Ba.1 variant SARS-CoV-2 pseudovirus - #241129".
Intended use	For Research Use Only. Not for Use in Diagnostic Procedures. Not Meant for Resale.