



**SARS-CoV-2 Pseudovirus  
Omicron (BA.1) Variant**

**Luciferase reporter**

**Lot #251215-BA1-30**



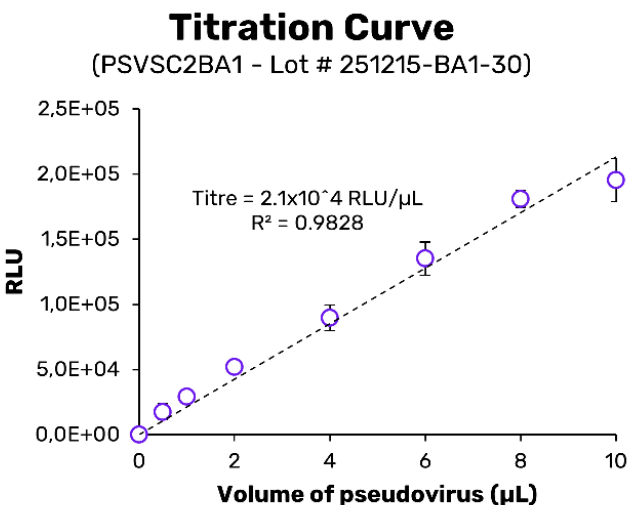
**Certificate of Analysis**

# 1. Summary

This certificate provides a functional validation of the SARS-CoV-2 pseudovirus, Omicron (BA.1), lot #251215-BA1-30. The titer is  $2.1 \times 10^4$  RLU/ $\mu$ L. A 1,000-fold-to-background ratio is obtained with 1  $\mu$ L per well (96-well plate). Hence, a volume of 1 mL 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<sup>+</sup>)  
**Volume of pseudovirus** 0 – 0.5 – 1 – 2 – 4 – 6 – 8 – 10  $\mu$ L/well  
**Detection signal** Luminescence (firefly luciferase)  
**Detection method** Microplate reader Biotek Synergy H1 (Gain: 135)



Pseudovirus ( $\mu$ L)	RLU 1	RLU 2	Mean	CV	Fold to Background
0	2,40E+01	2,80E+01	2,60E+01	2,83E+00	1,00E+00
0,5	1,32E+04	2,17E+04	1,75E+04	6,00E+03	6,72E+02
1	2,72E+04	3,13E+04	2,92E+04	2,90E+03	1,12E+03
2	5,20E+04	5,18E+04	5,19E+04	1,27E+02	2,00E+03
4	8,26E+04	9,67E+04	8,97E+04	9,93E+03	3,45E+03
6	1,26E+05	1,44E+05	1,35E+05	1,26E+04	5,20E+03
8	1,86E+05	1,77E+05	1,81E+05	6,33E+03	6,96E+03
10	2,07E+05	1,84E+05	1,96E+05	1,65E+04	7,52E+03

### Figure 1: Transduction efficiency curve

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

### Conclusion

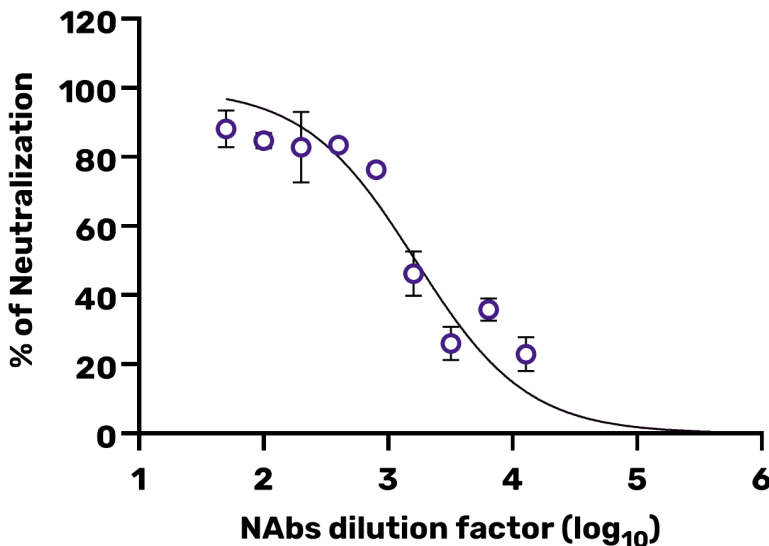
The SARS-CoV-2 pseudovirus, Omicron (BA.1) variant (lot #251215-BA1-30), is capable of transducing target cells. The titer is  $2.1 \times 10^4$  RLU/ $\mu$ L. Using 1 microliters of pseudovirus per reaction in a 96-well plate results in a 1,000-fold increase in RLU compared to the background. Accordingly, 1 mL of lot #251215-BA1-30 can be used to perform approximately 1,000 reactions, or  $10 \times$  96-well plates, according to IVANO Bioscience’s protocol (available upon request)

### 3. Neutralization assay

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

#### Neutralization curve

(PSVSC2BA1 - lot #251215BA1-30)



**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  $\mu$ L of complete medium and incubated for 1 hour at 37 °C, with 1  $\mu$ L of pseudovirus, in a 96-well plate. Then, an additional 50  $\mu$ L containing 10,000 cells was seeded in each well and incubated for 72 hours. Finally, an additional 100  $\mu$ 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  $\mu$ 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 SARS-CoV-2 pseudovirus, Omicron (BA.1) variant (lot #251215-BA1-30) can be efficiently neutralized by neutralizing antibodies.

## 4. Additional information

<b>Intruction of use</b>	We recommend determining the titer in your lab's conditions before performing any experiments Handle under biosafety level-2
<b>Pseudovirus</b>	3 <sup>rd</sup> generation lentiviral vector, incompetent replication and non-toxic
<b>Pseudotyping</b>	Spike glycoprotein of the SARS-CoV-2 pseudovirus, Omicron (BA.1) variant GISAID Accession Number: EPI_ISL_10866182
<b>AA Substitutions</b>	Spike A67V, Spike D614G, Spike D796Y, Spike E484A, Spike G142D, Spike G339D, Spike G446S, Spike G496S, Spike H69del, Spike H655Y, Spike ins214EPE, Spike K417N, Spike L212I, Spike L981F, Spike N211del, Spike N440K, Spike N501Y, Spike N679K, Spike N764K, Spike N856K, Spike N969K, Spike P681H, Spike Q493R, Spike Q498R, Spike Q954H, Spike S371L, Spike S373P, Spike S375F, Spike S477N, Spike T95I, Spike T478K, Spike T547K, Spike V70del, Spike V143del, Spike Y144del, Spike Y145del, Spike Y505H
<b>Pseudotyping sequence</b>	MFVFLVLLPLVSSQC VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDL FLPFFSNVTWFHVISGTNGTKRFDNPVLPFNDGVYFASIEKSNIRGWIFGTTLDS KTQSLLVN NATNVVIK VCEFCNDPFLDHKNNKSWMESEFRVYSSANNCTFE YVSQPFLMDLEGKQGNFKNLREFVFNIDGYFKIYSKHTPIIVRDLPQGFSALEP LVDLPIGINITRFQTL LALHRSYLT PGDSSSGWTAGAAAYYVGYLQRPRTFLLKYN ENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLC PFDEVFNATRFASVYAWNRKRISNCVADYSVLYNLAPFFFTFKCYGVSPTKLNDL CFTNVYADSFVIRGDEVRQIAPGQTGNIADYNYKLPDDFTGCVIAWNSNKLDSK VSGNYNYLYRLFRKSNLKPFERDISTEIQAGNKP CNGVAGFNCYFPLRSYSFR PTYGVGHQPYRVVVL SFELLHAPATVCGPKKSTNLVKNKCVNFNFGLKGTGV L TESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQ VAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEYVNNSEYE CDIPIGAGICASYQTQTKSHSRASSVASQSIIAYTMSLGAENSVAYSNNNSIAIPTN FTISVTTEILPVSMTKTSVDCTMYICGDSTEC SNLLLQYGSFCTQLKRALTGIAVE QDKNTQEVFAQVKQIYKTPPIKYFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLA DAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSG WTFGAGAALQIPFAMQMAYRFNGIGV TQNVLYENQKLIANQFN SAIGKIQDLSL STASALGKLQDVVNHNAQALNTLVKQLSSKFGAISSVLNDIFSRDLKVEAEVQID RLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGGQSKRVDFCGKGYHL MSFPQSAPHGVVFLHVITYVPAQEKNFTTAPAICHDGKAHFPREGVFSNGTHW FVTQRNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKN HTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPW YIWLGFIAGLIAIVMVTIMLCCMTSCC SCLKGCCSCGSCC
<b>Glycosylation origin</b>	Human
<b>Reporter Protein</b>	Firefly luciferase
<b>Storage</b>	- 80 °C, avoid freeze/thaw cycles
<b>For more information</b>	mathias.mangion@ivanobioscience.com Message object should contain: "PSVSC2B117– lot #251215-BA1-30"
<b>Intended use</b>	For Research Use Only Not for Use in Diagnostic Procedures, not Meant for Resale