IVANO BIOSCIENCE

SARS-CoV-2 Delta Pseudovirus

B.1.617.2

Luciferase reporter

Lot #240624

Certificate of Analysis

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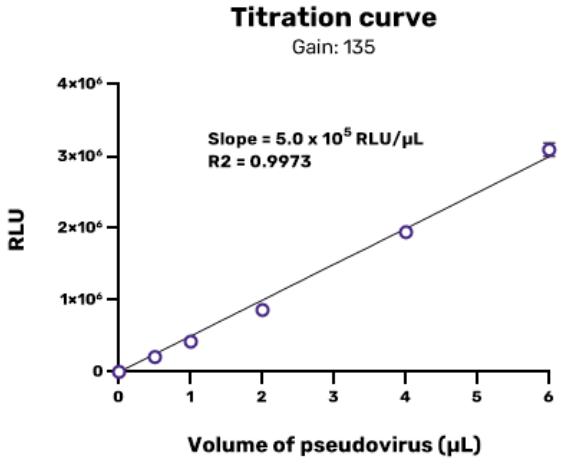
1. Summary

This certificate is a functional validation for the lot #240624 of a Spike glycoprotein of the SARS-CoV-2 B.1.617.2 (Delta) variant. The titer is 5,0 x 10^5 RLU/µL. A volume of 1 mL of lot #240624 can be used to perform approximately 2,000 reactions or 20 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 - 0.5 – 1 – 2 – 4 – 6 – 8 – 10 µL/well
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)



Volume of pseudovirus (µL)	Raw Data Mean	Fold *vs* Background
0	1,28E+02	Background
0,5	2,08E+05	1,63E+03
1	4,21E+05	3,30E+03
2	8,61E+05	6,75E+03
4	1,94E+06	1,52E+04
6	3,09E+06	2,42E+04
8	OVRFLW	-
10	OVRFLW	-

Figure 1: Transduction efficiency curve
A volume range of pseudoviruses was mixed in a final volume of 50 µL of culture complete medium, in a 96-well plate. Then, an additional 50 µL containing 10 000 cells was seeded in each well. Luc expression analysis was performed 72 hours post-infection by a luminescence microplate reader.

Conclusion:

The SARS-CoV-2 Delta B.1.617.2 pseudovirus (#240624) can transduce the target cells. The titer is: 5,0 x 10^5 RLU/µL. Using 0.5 µL/reaction of pseudovirus in a 96-well plate will yield a 1,000-fold increase in RLU compared to the background. Therefore, 1 mL of lot #240624 can be used to perform approximately 2,000 reactions or 20 x 96-well plates (according to the IVANO Bioscience protocol available upon request).

Note that volumes of 8 and 10 µL of pseudovirus provided an overflow signal upon analysis.

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)

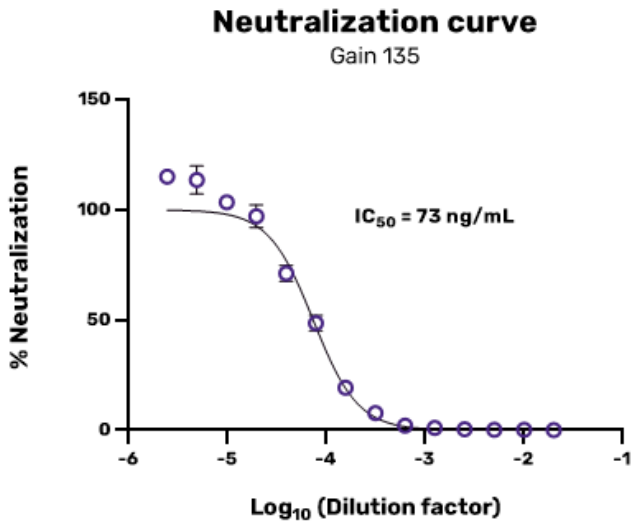


Figure 2: Neutralization curve

A monoclonal neutralizing antibody (Ab02019-12.1), at a starting dilution of 20 µg/mL, was serial 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 SARS-CoV-2 Delta B.1.617.2 pseudovirus (lot #240624) can be efficiently neutralized by neutralizing antibodies.

4. Additional information

Instruction of Uses	We recommend determining the titer in your lab's conditions before performing any experiments. Handle under biosafety level-2.
Pseudovirus	Replication incompetent. 3rd generation lentiviral vector, incompetent replication and non-toxic.
Pseudotyping	Spike glycoprotein of the SARS-CoV-2 Delta B.1.617.2 variant. Derived from the original sequence (GenBank: MN908947) with multiple mutations including: T19R, del157/158, L452R, T478K, D614G, P681R, D950N, The spike protein has an 18-aa cytoplasmic tail truncation for optimal infection. See sequence below: MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI HVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCN DPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIY SKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQP RTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVF NATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQ IAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGST PCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFN GLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQ GVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSP SRASSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSN LLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDL LFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGA GAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQA LNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATK MSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGV FVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSP DVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIM LCCMTSCCSCLKGCCSCGSCC
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: " SARS-CoV-2 Delta- #240624".
Intended use	For Research Use Only. Not for Use in Diagnostic Procedures. Not Meant for Resale.