IVANO BIOSCIENCE

SARS-CoV-2 Pseudovirus

Delta B.1.617.2 variant

Luciferase reporter

Lot #250731

Certificate of Analysis

August 4, 2025

Version 1

1. Summary

This certificate is a functional validation for the SARS-CoV-2 pseudovirus, Delta B.1.617.2 variant, lot #250731. The titer is 5.01 x 10^5 RLU/µL. A volume of 1 mL can be used to perform 1,000 reactions or 10 x 96-well plates, according to IVANO Bioscience's protocol, available upon request.
The long-term stability of this pseudovirus is expected to be at least 1 year at -80°C, without freeze/thaw cycle.

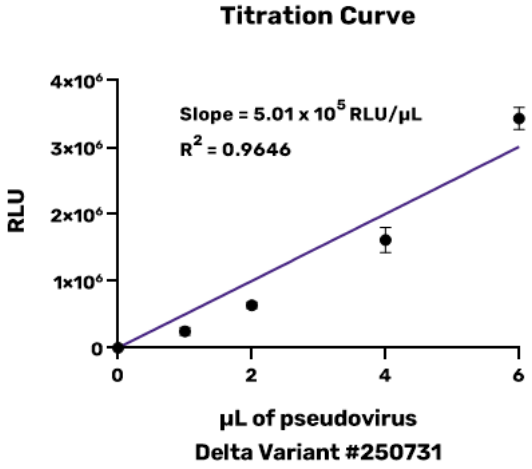
2. Transduction efficiency assay

Target cells HEK293 cells ($ACE2^+$)

Volume of pseudovirus 0 – 0.5 – 1 – 1.5 – 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)	RLU1	RLU2	Mean	Fold to Background
0	210	173	191.5	-
1	2.09E+05	2.88E+05	2.48E+05	1.30E+03
2	6.72E+05	6.05E+05	6.39E+05	3.33E+03
4	1.48E+06	1.75E+06	1.62E+06	8.44E+03
6	3.31E+06	3.55E+06	3.43E+06	1.79E+04
8	OVRFLW	OVRFLW	-	-
10	OVRFLW	OVRFLW	-	-

Figure 1: Transduction efficiency curve
A volume range of pseudovirus was mixed in a final volume of 50 µL of medium, in a 96-well plate. Then, 50 µL of medium containing 10,000 cells was seeded in each well. On the day of analysis, an additional 100 µL 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 µL of the cell lysate, using a microplate reader. Data are expressed in relative unit luminescence (RLU).

Conclusion

The SARS-CoV-2 pseudovirus, Delta B.1.617.2 variant (#250731) can transduce the target cells. The titer is 5.01 x 10^5 RLU/µL. Using 1 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 #250731 could be used to perform approximately 1,000 reactions or 10 x 96-well plates, according to IVANO Bioscience's protocol (available upon request). Note that starting from a volume of 8 µL of pseudovirus, an overflow signal is observed upon analysis.

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3. Neutralization assay

Target cells	HEK293 cells ($ACE2^+$)
Volume of pseudovirus	1 µL / well
Neutralizing antibody (Nabs)	Anti-Spike Protein (RBD) [CV30], Ab02012-12.1
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)

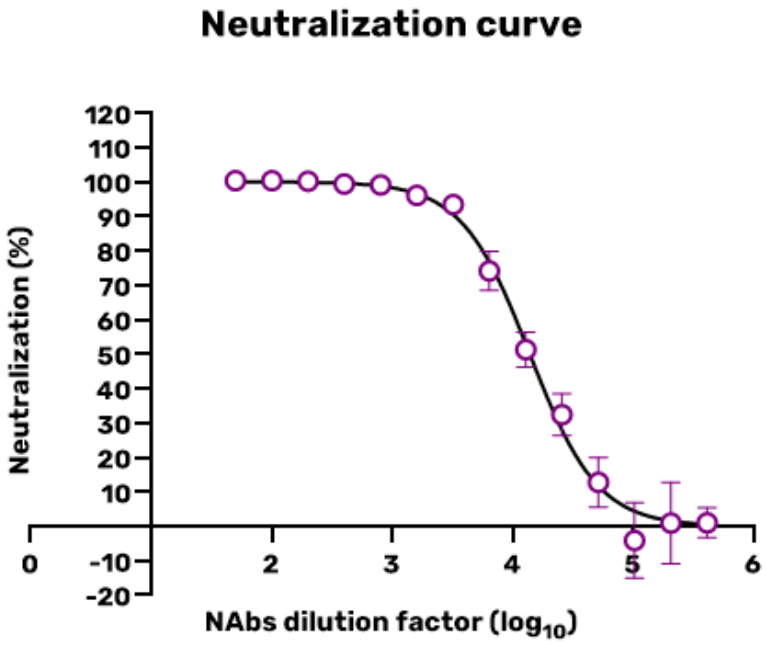

Figure 2: Neutralization curve
A starting dilution of 1/50 of a neutralizing antibody was serially diluted in a final volume of 50 µL of medium and incubated for 1 hour at 37 °C, with 1 µL of pseudovirus, in a 96-well plate. Then, 50 µL of medium, containing 10,000 cells, was seeded in each well and incubated for 72 hours. On the day of analysis, an additional 100 µL 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 µL of the cell lysate, using 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, Delta B.1.617.2 variant (#250731) can be efficiently neutralized by neutralizing antibodies.

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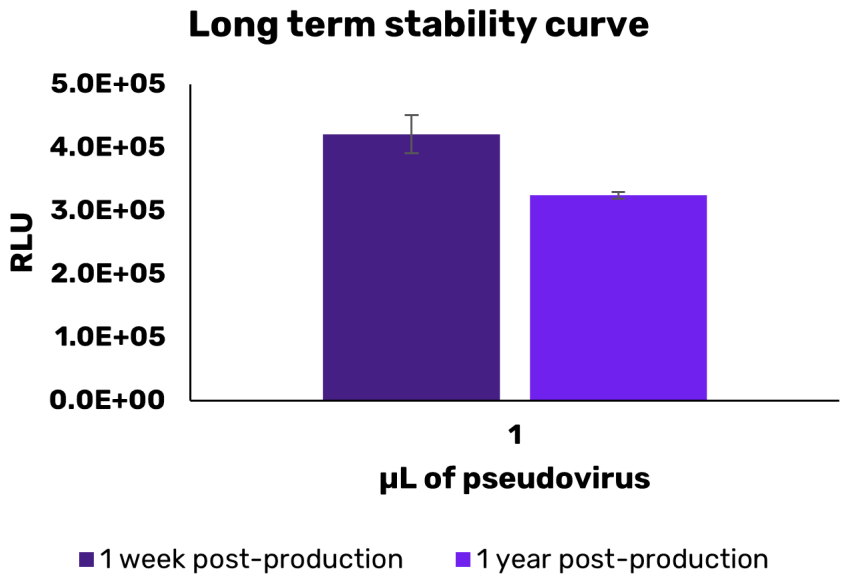
4. Long-term stability assay

Target cells	HEK293 cells (ACE2^{+})
Volume of pseudovirus	1 µL/well
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)



1 week post-production				1 year post-production			
Volume of pseudovirus (µL)	Mean	CV	Fold to Background	Volume of pseudovirus (µL)	Mean	CV	Fold to Background
0	1.28E+02	1.20E+01	1.00E+00	0	3.65E+02	1.51E+02	1.00E+00
0.5	2.08E+05	1.11E+04	1.63E+03	0.5	1.58E+05	2.76E+03	4.34E+02
1	4.21E+05	3.03E+04	3.30E+03	1	3.24E+05	5.22E+03	8.88E+02
2	8.61E+05	5.07E+04	6.75E+03	2	6.86E+05	6.16E+04	1.88E+03
4	1.94E+06	6.17E+04	1.52E+04	4	2.42E+06	4.51E+04	6.64E+03

Figure 3: Long-term stability curve
This analysis was carried out using lot #240624, 1 week and 1 year after production. 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).

Conclusion

The results indicate that the pseudovirus lot #240624 can be used at least 1 year post-production, while maintening a fold to background neat 10^{3}. As production lot #240624 is stable at -80°C for at least 1 year, we expect production lot #250731 to show comparable stability.

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5. 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

3rd generation lentiviral vector, incompetent replication and non-toxic.

Pseudotyping

Spike glycoprotein of the SARS-CoV-2 Delta B.1.617.2 variant

Pseudotyping sequence

MFVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDL
FLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASIEKSNIIRGWIFGTTL
DSKTQSLLIVNNATNVVIKVCEFQFCNDPFLDVYYHKNNKSWMESGVYSSANNC
TFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFS
ALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLL
KYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNIT
NLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKL
NDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNL
DSKVGGNYNYRYRLFRKSNLKPFERDISTEIYQAGSKPCNGVEGFNCYFPLQSY
GFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTG
TGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNT
SNQVAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVN
NSYECDIPIGAGICASYQTQTNSRSRASSVASQSIIAYTMSLGAENSVAYSNNSIA
IPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTG
IAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNK
VTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGT
ITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQD
SLSSTASALGKLQNVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEV
QIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKG
YHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNG
THWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDK
YFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYI
KWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCC

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 pseudovirus – #250731".

Intended use

For Research Use Only. Not for Use in Diagnostic Procedures. Not Meant for Resale.

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