IVANO BIOSCIENCE

SARS-CoV-2 Pseudovirus

D614G variant

Luciferase reporter

Lot #240906

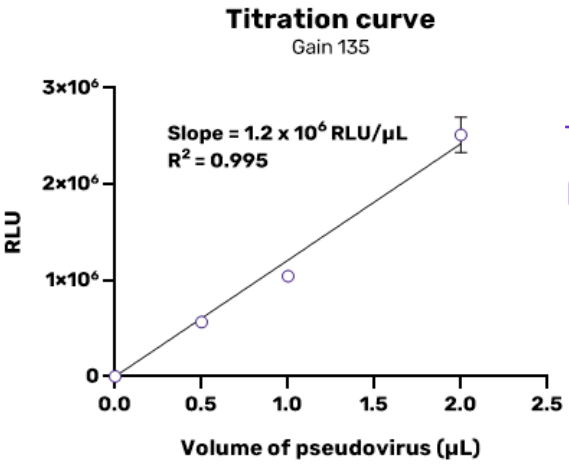
Certificate of Analysis

1. Summary

This certificate is a functional validation for the lot #240906 of the D614G variant SARS-CoV-2 pseudovirus. The titer is 1.2 x 10^6 RLU/µL. A volume of 1 mL can be used to perform between 1,000 and 2,000 reactions or 10 to 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 – 1.5 - 2 – 4 µL/well
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)



Volume of pseudovirus (µL)	RLU 1	RLU 2	Mean RLU	CV RLU	Fold vs Background
0	4.7E+02	5.7E+02	5.2E+02	7.0E+01	1.0E+00
0.5	5.5E+05	5.8E+05	5.7E+05	2.4E+04	1.1E+03
1	1.0E+06	1.1E+06	1.0E+06	2.2E+04	2.0E+03
2	2.4E+06	2.6E+06	2.5E+06	1.8E+05	4.9E+03
4	OVRFLW	OVRFLW	-	-	-

Figure 1: Transduction efficiency curve
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 D614G variant SARS-CoV-2 pseudovirus (#240906) can transduce the target cells. The titer is: 1.2 x 10^6 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 background. Therefore, 1 mL of lot #240906 could 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 a volume of 4 µL of pseudovirus provided an overflow signal upon analysis.

3. Neutralization assay

Target cells	HEK293 cells (ACE2⁺, TMPRSS2⁺)
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)

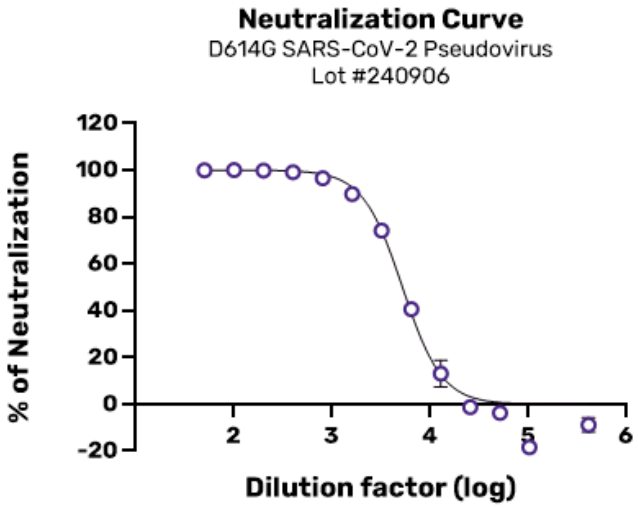


Figure 2: Neutralization curve

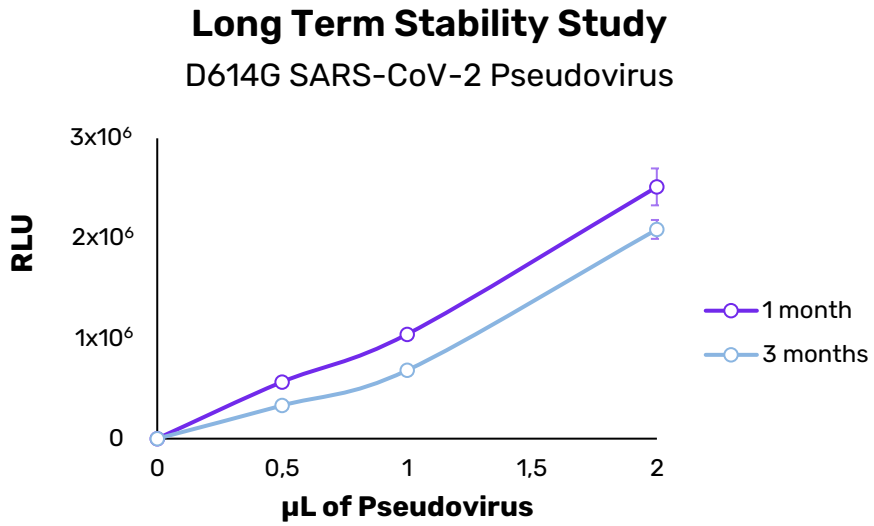
A monoclonal neutralizing antibody (Ab02019-12.1), at a starting dilution of 20 µg/mL, was serial diluted in a final volume of 50 µL of complete medium and incubated for 1 hour at 37 °C, with 1 µL of pseudovirus, in a 96-well plate. Then, an additional 50 µL containing 10 000 cells was seeded in each 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 D614G variant SARS-CoV-2 pseudovirus (#240906) can be efficiently neutralized by neutralizing antibodies.

4. Long term stability assessment

Target cells	HEK293 cells (ACE2⁺, TMPRSS2⁺)
Volume of pseudovirus	0 - 0.5 – 1 – 1.5 - 2 – 4 µL/well
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)



1 month				
µL of pseudovirus	**RLU 1**	**RLU 2**	**Mean**	**CV**
0	4.7E+02	5.7E+02	5.2E+02	7.0E+01
0.5	5.5E+05	5.8E+05	5.7E+05	2.4E+04
1	1.0E+06	1.1E+06	1.0E+06	2.2E+04
2	2.4E+06	2.6E+06	2.5E+06	1.8E+05

3 months				
µL of pseudovirus	**RLU 1**	**RLU 2**	**Mean**	**CV**
0	8.2E+01	8.2E+01	8.2E+01	0.0E+00
0.5	3.6E+05	3.1E+05	3.3E+05	3.4E+04
1	7.0E+05	6.6E+05	6.8E+05	3.1E+04
2	2.2E+06	2.0E+06	2.1E+06	9.4E+04

Figure 1: Long term stability curve
This analysis was carried out using two vials of the same production batch. 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:
There is no significant difference in transduction efficiency between the two conditions evaluated. This batch is stable for at least 3 months.

5. Freeze / thaw stability assessment

Target cells	HEK293 cells ($ACE2^+$, $TMPRSS2^+$)
Volume of pseudovirus	1 µL/well
Detection signal	Luminescence (firefly luciferase)
Detection method	Microplate reader Biotek Synergy H1 (Gain: 135)

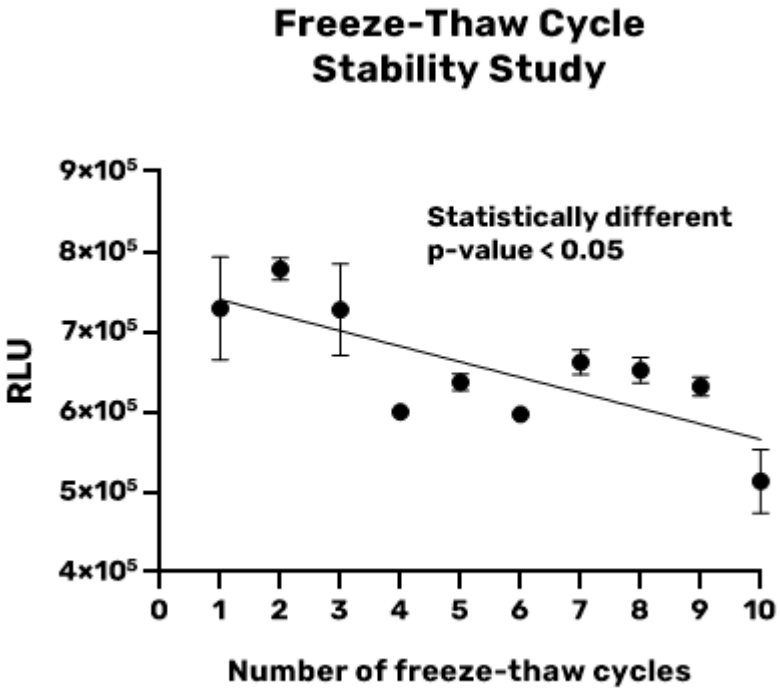


Figure 1: Freeze/thaw cycle transduction efficiency
This analysis was carried out using 10 vials of the same production batch where each vial was freezed/thawed between one and ten times. Then, a volume range of pseudovirus was mixed in a final volume of 50 µL of transduction medium, in a 96-well plate. 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 = 0.0003). However, 70% of the transduction efficiency is maintained after 10 free-thaw cycles with a high transduction efficiency of 10^5 RLU/µL.

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	3rd generation lentiviral vector, incompetent replication and non-toxic.
Pseudotyping	Spike glycoprotein of the D614G variant SARS-CoV-2. 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: " D614G variant SARS-CoV-2 pseudovirus - #240906".
Intended use	For Research Use Only. Not for Use in Diagnostic Procedures. Not Meant for Resale.