

CLAIMS

1. A pseudovirus encoding:
 - (i) a SARS-CoV-2 Spike (S) glycoprotein;
 - (ii) one or more of Rev, Tat, Gag, and Pol proteins; and
 - (iii) a reporter protein.
2. The pseudovirus of claim 1, encoding from 1 to 20, from 2 to 20, from 3 to 20, from 4 to 20, from 5 to 20, from 6 to 20, from 7 to 20, from 8 to 20, 9 to 20, from 10 to 20, from 11 to 20, from 12 to 20, from 13 to 20, from 14 to 20, from 15 to 20, from 16 to 20, from 17 to 20, from 18 to 20, from 19 to 20, from 1 to 10, from 2 to 10, from 3 to 10, from 4 to 10, from 5 to 10, from 6 to 10, from 7 to 10, from 8 to 10, from 2 to 5, from 3 to 5, from 4 to 8, from 5 to 8, or from 6 to 8 SARS-CoV-2 S glycoproteins.
3. The pseudovirus of claim 1, wherein the reporter protein is at least one of luciferase, a fluorescent protein and ZsGreen.
4. The pseudovirus of any one of claims 1-3, wherein the SARS-CoV-2 S glycoprotein has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to a polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.
5. The pseudovirus of any one of claims 1-3, wherein the SARS-CoV-2 S glycoprotein has an inactive furin cleavage site.
6. The pseudovirus of claim 5, wherein the SARS-CoV-2 S glycoprotein has an inactive furin cleavage site having the amino acid sequence of QQAQ (SEQ ID NO: 68).
7. The pseudovirus of any one of claims 1-3, wherein amino acids 973 and 974 of the SARS-CoV-2 S glycoprotein are proline, as compared to a wild-type SARS-CoV-2 S glycoprotein having the amino acid sequence of SEQ ID NO: 2.
8. The pseudovirus of any one of claims 1-3, wherein the SARS-CoV-2 S glycoprotein has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to a polypeptide of any one of SEQ ID NOS: 3, 5-13, 15, 17-19, 21, and 23-66.

9. The pseudovirus of any one of claims 1-3, wherein the SARS-CoV-2 S glycoprotein has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to a polypeptide of any one of SEQ ID NOS: 3-13, 20, and 22-66.
10. The pseudovirus of any one of claims 1-3, wherein the SARS-CoV-2 S glycoprotein has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to a polypeptide of any one of SEQ ID NOS: 3, 5-13, and 23-66.
11. The pseudovirus of any one of claims 1-3, wherein the SARS-CoV-2 S glycoprotein is from a SARS-CoV-2 virus or a variant of SARS-CoV-2.
12. The pseudovirus of claim 11, wherein the variant of SARS-CoV-2 is a B.1.1.7 SARS-CoV-2 strain; a B.1.351 SARS-CoV-2 strain; a P.1 SARS-CoV-2 strain; a Cal.20C SARS-CoV-2 strain; a B.1.617.2 SARS-CoV-2 strain; a B.1.525 SARS-CoV-2 strain; a B.1.526 SARS-CoV-2 strain; a B.1.617.1 SARS-CoV-2 strain; a C.37 SARS-CoV-2 strain; a B.1.621 SARS-CoV-2 strain; or a B.1.1.529 SARS-CoV-2 strain.
13. The pseudovirus of any one of claims 1-3; wherein the SARS-CoV-2 S glycoprotein has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to a SARS-CoV-2 S glycoprotein from a SARS-CoV-2 S omicron variant selected from the group consisting of: BA.1, BA.2.12.1, BA.2, BAJ, BA.4, BA.5, XBB.1.5, XBB.2.3, and XBB.1.16.
14. The pseudovirus of any one of claims 1-3, comprising a Gag protein and a Pol protein.
15. The pseudovirus of claim 14, wherein a fusion protein encodes the Gag protein and Pol protein.
16. The pseudovirus of claim 14, wherein the Gag protein and Pol protein are from a Murine Leukemia Virus (MLV).

17. The pseudovirus of any one of claims 1-3, wherein the one or more of Rev, Tat, Gag, and Pol proteins are from a lentivirus.
18. A method of producing the pseudovirus of any one of claims 1-3, comprising transfecting a host cell with:
- (i) a transfer plasmid encoding a reporter protein;
 - (ii) one or more plasmids encoding a Rev protein, a Tat protein, a Gag protein, a Pol protein; or a combination thereof; and
 - (iii) a plasmid encoding a SARS-CoV-2 Spike (S) glycoprotein.
19. The method of claim 18, wherein the host cell is human.
20. The method of claim 18, wherein the host cell is a HEK293T cell.
21. A method for determining if a biological sample contains neutralizing antibodies against a SARS-CoV-2 virus or a variant thereof, comprising:
- (a) contacting a biological sample with from 1 to about 20 pseudoviruses of any one of claims 1-3;
 - (b) contacting a cell expressing angiotensin converting enzyme 2 (ACE2) with the from 1 to about 20 pseudoviruses;
 - (c) quantifying expression of the reporter protein in the cell expressing ACE2;
 - (d) contacting a control cell expressing ACE2 with the from 1 to about 20 pseudoviruses, wherein the from 1 to about 20 pseudoviruses have not been contacted with the biological sample; and
 - (e) quantifying expression of the reporter protein in the control cell expressing ACE2;
- wherein if the expression of the reporter protein in the cell of (c) is less than expression of the reporter protein in the control cell of (e), then the biological sample contains neutralizing antibodies against the SARS-CoV-2 virus or the variant thereof.
22. The method of claim 21, comprising contacting the biological sample and pseudovirus for from 1 hour to 7 hours.
23. The method of claim 22, comprising contacting the biological sample and pseudovirus for two hours.

24. The method of claim 21, comprising contacting the biological sample and pseudovirus at about 37 °C.
25. The method of claim 21, further comprising heat inactivating the biological sample.
26. The method of claim 21, comprising contacting the cell with pseudovirus for from 1 day to 7 days.
27. The method of claim 21, comprising contacting the cell with pseudovirus for about 3 days.
28. The method of claim 21, wherein the cells are selected from the group consisting of: Vero E6, A549, and HEK293T cells.
29. The method of claim 28, wherein the cells are HEK293T cells.
30. The method of claim 21, wherein performing the method does not require a biosafety level 3 (BSL-3) laboratory.
31. The method of claim 21, wherein the biological sample is serum, plasma, or blood obtained from a patient that had previously been administered an immunogenic composition against a SARS-CoV-2 virus or a variant thereof.
32. The method of claim 21, wherein the biological sample is serum, plasma, or blood obtained from a patient that had previously had COVID-19.
33. The method of claim 21, comprising contacting the biological sample with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 pseudoviruses.
34. The method of claim 33, wherein each pseudovirus expresses a different reporter gene and a different SARS-CoV-2 S glycoprotein.