Simple Protocol for PSV-Based Neutralization Assay

SARS-CoV-2 (2019nCoV) pseudotype virus (pseudovirus, PSV) for COVID-19 related vaccines and neutralizing antibodies evaluation

The outbreak of COVID-19, caused by SARS-CoV-2 (2019-nCoV), has been a global public health threat and caught the worldwide concern. Due to its high pathogenicity and infectivity[1], live SARS-CoV-2 should be handled under biosafety level 3 (BSL-3) conditions. GeneMedi has developed SARS-CoV-2 pseudovirus production system, from which the SARS-CoV-2 pseudotyped virus can be handled in biosafety level 2 (BSL-2)[2].

GeneMedi's SARS-CoV-2 (2019nCoV) pseudotype virus (pseudovirus, PSV) based neutralization assay is a standard evaluation procedure for COVID-19 related vaccines and neutralizing antibodies potency evaluation. GeneMedi's SARS-CoV-2 PSV is the core ingredient of diagnostics for neutralization serology after vaccinotherapy.

GeneMedi's SARS-CoV-2 pseudotyped virus includes wildtype and the spike mutation variants (D614G, S943P, V367F, G476S, V483A, H49Y, Q239K, A831V, P1263L, D839Y/N/E: D839Y, D839N, D839E). The GeneMedi's SARS-CoV2 PSV panel help for all-in-one vaccinotherapy evaluation.

Application

SARS-CoV-2(2019nCoV) Pseudotyped Virus Based Neutralization Assay[3]

Coronavirus disease 2019 (COVID-19) pandemic is caused by SARS-CoV-2 (2019nCoV) infection, a newly emerged novel coronavirus spreading worldwide. Current efforts are focusing on development of specific antiviral drugs. Therapeutic neutralizing antibodies (NAbs) against SARS-CoV-2(2019-nCoV) will be greatly important therapeutic agents for the treatment of COVID-19. The availability of therapeutic NAbs against SARS-CoV-2 will offer benefits for the control of the current pandemic and the possible re-emergence of the virus in the future, and their development therefore remains a high priority.

GeneMedi's NAbs has been validated to reduce SARS-CoV-2 lentivirus-based pseudo virus infectivity and thereby blocking the entry of the Coronavirus to its effector/targeting cell: human ACE2-HEK293T cell (hACE2-HEK293T, Cat. GM-SC-293T-hACE201). GeneMedi's SARS-CoV-2 (2019nCoV) Nabs can act as a benchmark of neutralizing antibodies discovery against COVID-19.

Tel: +86-21-50478399
Email: support@genemedi.net

GeneMedi's Pseudovirus Based Neutralization Assay (PBNA) is a conventional assay method that is suitable for High-Throughput Screening (HTS) without live virus engaged. The Pseudovirus Based Neutralization Assay can be used for evaluating

1) Neutralizing antibodies (NAbs)[3,4]

2) Peptides blockers[5,6] (peptide inhibitors) or protein[7,8]

3) Types of Vaccines (Immunized serum)[9]

4) Compounds targeting Spike induced cell-fusion[10].

Materials

1. SARS-CoV-2 Pseudovirus-RFP-fLuciferase (GM-2019nCoV-PSV01)
2. Effecter cell: Alternative
   A. hACE2-HEK293T stable cell line (GM-SC-293T-hACE2-01)
   B. Wildtype HEK293T cell line, hACE2 vector for transfection (GMV-V-2019nCoV-041)
3. Neutralizing antibodies (NAbs) (GMP-V-2019nCoV-SnAb001~GMP-V-2019nCoV-SnAb005)

Pseudotyped virus of SARS-CoV-2 Spike Mutation Variants (D614G, S943P, V367F, G476S, V483A, H49Y, Q239K, A831V, P1263L, D839Y/N/E:D839Y, D839N, D839E)

Catalog No.	Pseudotyped virus of SARS-CoV-2 Spike Mutation Variants
GM-2019nCoV-PSV02	Spike D614G mutation SARS-CoV-2(2019nCoV) Pseudotyped virus
GM-2019nCoV-PSV03	Spike S943P mutation SARS-CoV-2(2019nCoV) Pseudotyped virus
GM-2019nCoV-PSV04	Spike V367F mutation SARS-CoV-2(2019nCoV) Pseudotyped virus
GM-2019nCoV-PSV05	Spike G476S mutation SARS-CoV-2(2019nCoV) Pseudotyped virus
GM-2019nCoV-PSV06	Spike V483A mutation SARS-CoV-2(2019nCoV) Pseudotyped virus
GM-2019nCoV-PSV07	Spike H49Y mutation SARS-CoV-2(2019nCoV) Pseudotyped virus
GM-2019nCoV-PSV08	Spike Q239K mutation SARS-CoV-2(2019nCoV) Pseudotyped virus
GM-2019nCoV-PSV09	Spike A831V mutation SARS-CoV-2(2019nCoV) Pseudotyped virus
GM-2019nCoV-PSV10	Spike P1263L mutation SARS-CoV-2(2019nCoV) Pseudotyped virus
GM-2019nCoV-PSV11	Spike D839Y/N/E-D839Y mutation SARS-CoV-2(2019nCoV) Pseudotyped virus
GM-2019nCoV-PSV12	Spike D839Y/N/E-D839N mutation SARS-CoV-2(2019nCoV) Pseudotyped virus
GM-2019nCoV-PSV13	Spike D839Y/N/E-D839E mutation SARS-CoV-2(2019nCoV) Pseudotyped virus

Protocol:

If your effecter cell is hACE2-HEK293T stable cell line, please begin in Step 2.

1. Transfect HEK293T with hACE2-GFP vector (GMV-V-2019nCoV-041) 24hrs before planting the cell into 96-well.

2. Plant the hACE2-HEK293T into 96-well (5,000~10,000 per well) overnight before SARS-CoV-2 PSV infection.

3. Generation of 100ul PSV-Sample mixture:

100ul PSV-Sample mixture	Volume
GM-2019nCoV-PSV01*	50ul or 5ul
Sample(NAbs, peptides, serum, etc)	flexible (According to your own products)
Total	add culture medium to 100ul
* For GM-2019nCoV-PSV01-1, add 50ul in recommendation (range from20ul~100ul). For GM-2019nCoV-PSV01-2, add 5ul in recommendation. (range from2ul~10ul).	

Incubate PSV-Sample mixture for 1h at room temperature.

4. Remove the medium of effector cells in 96-well, add 100ul PSV-Sample mixture to 96-well for infection, 3 replicates per group.

5. Fluorescence imaging (RFP) 72hrs after SARS-CoV-2 PSV infection. The firefly luciferase reporter is measured following the Promega Luciferase Assay Reagent manual.

Tips

If your samples are serum

A standard curve should be generated using serially diluted Nabs (neutralizing antibodies) as a positive control.

If your samples are therapeutic antibodies or peptides candidates

Dilute the samples into concentration gradient for IC50 value evaluation.

Product information

Catalot Number	GM-2019nCoV-PSV01
Products Name	SARS-CoV-2 Pseudovirus-RFP-fLuciferase
Reporter	RFP+Firefly Luciferase
Ligand-Receptor	Spike-ACE2
Effector cell recommended	hACE2-HEK293T (Cat.GM-SC-293T-hACE201)
Size	0.5ml/vial
Products description	GeneMedi's SARS-CoV-2 Pseudovirus-RFP-fLuciferase (GM-2019nCoV-PSV01) is recombinant pseudotyped lentiviral particles containing SARS-CoV-2 spike protein to mimic SARS-CoV-2 (2019nCoV) cell infection and cell entry. The SARS-CoV-2 pseudovirus particles encode firefly luciferase and RFP in their lentiviral vector genome. The firefly luciferase and RFP gene will be strongly expressed after the SARS-CoV-2 pseudovirus entry into ACE2-expressing cells. Actually, 293T-hACE2(human ACE2 overexpression stable HEK293T cell lines) is normally used as effector cell (GM-SC-293T-hACE2-01). GM-2019nCoV-PSV01 is a powerful tool for SARS-CoV-2 related vaccine efficacy evaluation, neutralizing antibodies, peptides blockers competitors neutralization assay, and tissue-specific infection determination.
Bioactivity validation	Validated in hACE2-HEK293T Cell Entry
Storage	Store at -20℃ to -80℃ under sterile conditions. Avoid repeated freeze-thaw cycles.

GeneMedi SARS-CoV-2 Pseudovirus (PSV) Based Cell Entry

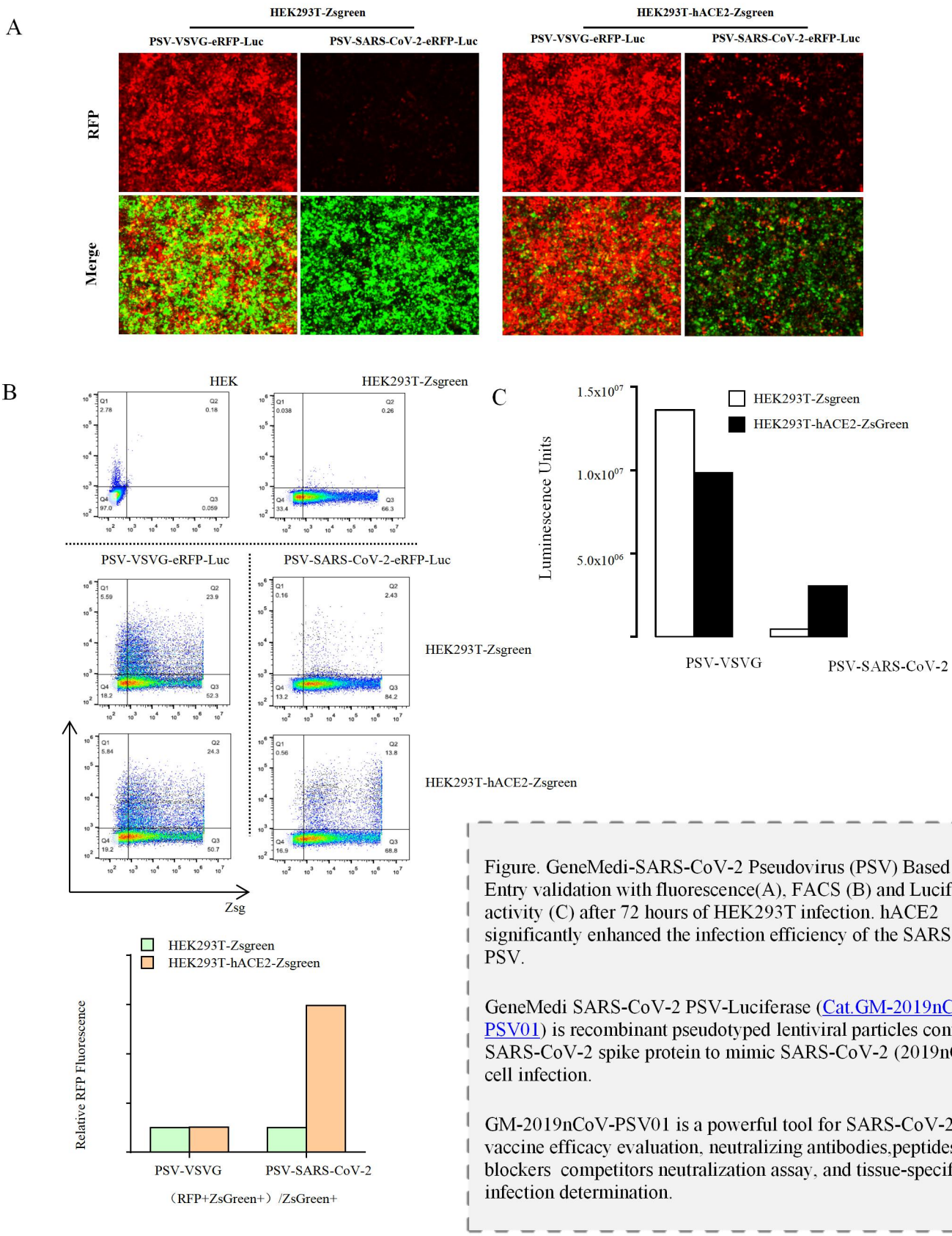


Figure. GeneMedi-SARS-CoV-2 Pseudovirus (PSV) Based Cell Entry validation with fluorescence(A), FACS (B) and Luciferase activity (C) after 72 hours of HEK293T infection. hACE2 significantly enhanced the infection efficiency of the SARS-CoV-2 PSV.

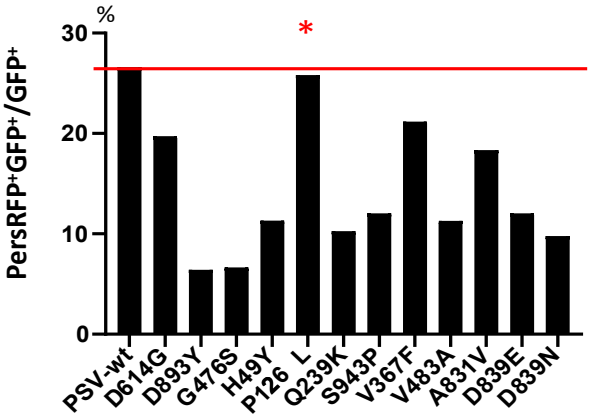
GeneMedi SARS-CoV-2 PSV-Luciferase (Cat.GM-2019nCoV-PSV01) is recombinant pseudotyped lentiviral particles containing SARS-CoV-2 spike protein to mimic SARS-CoV-2 (2019nCoV) cell infection.

GM-2019nCoV-PSV01 is a powerful tool for SARS-CoV-2 related vaccine efficacy evaluation, neutralizing antibodies,peptides blockers competitors neutralization assay, and tissue-specific infection determination.

--Nab discovery and vaccines evaluation through SARS-CoV-2 wildtype/mutant variants pseudovirus based neutralizing assay(PBNA) and Spike-ACE2 competition binding assay

GeneMedi-SARS-CoV-2 WT and Spike Mutation Variants Pseudovirus (PSV) Based Cell Entry

A



B

Name	RFP+GFP+/GFP+	Nor Ratio
PSV-wt	26.59	1.51
D614G	19.71	2.11
D893Y	6.41	1.00
G476S	6.67	1.48
H49Y	11.31	1.57
P1263L	25.82	1.29
Q239K	10.28	1.15
S943P	12.04	1.54
V367F	21.19	1.82
V483A	11.28	1.75
A831V	18.33	1.25
D839E	12.04	1.64
D839N	9.79	1.38

C

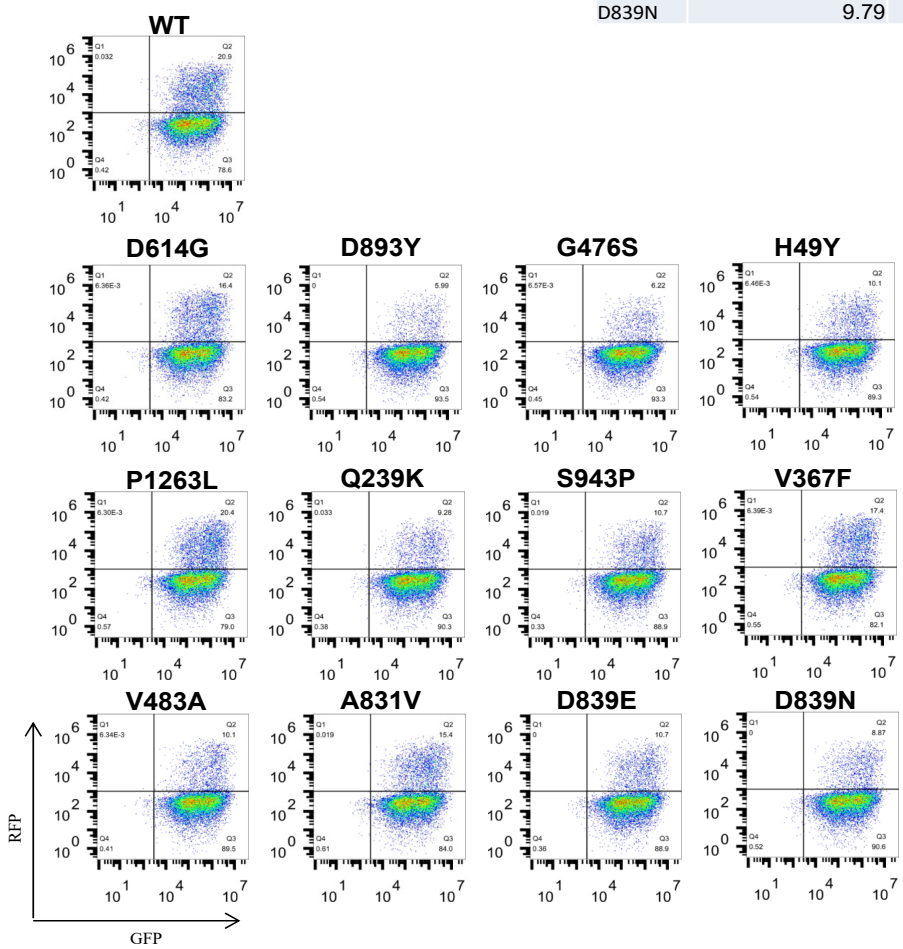
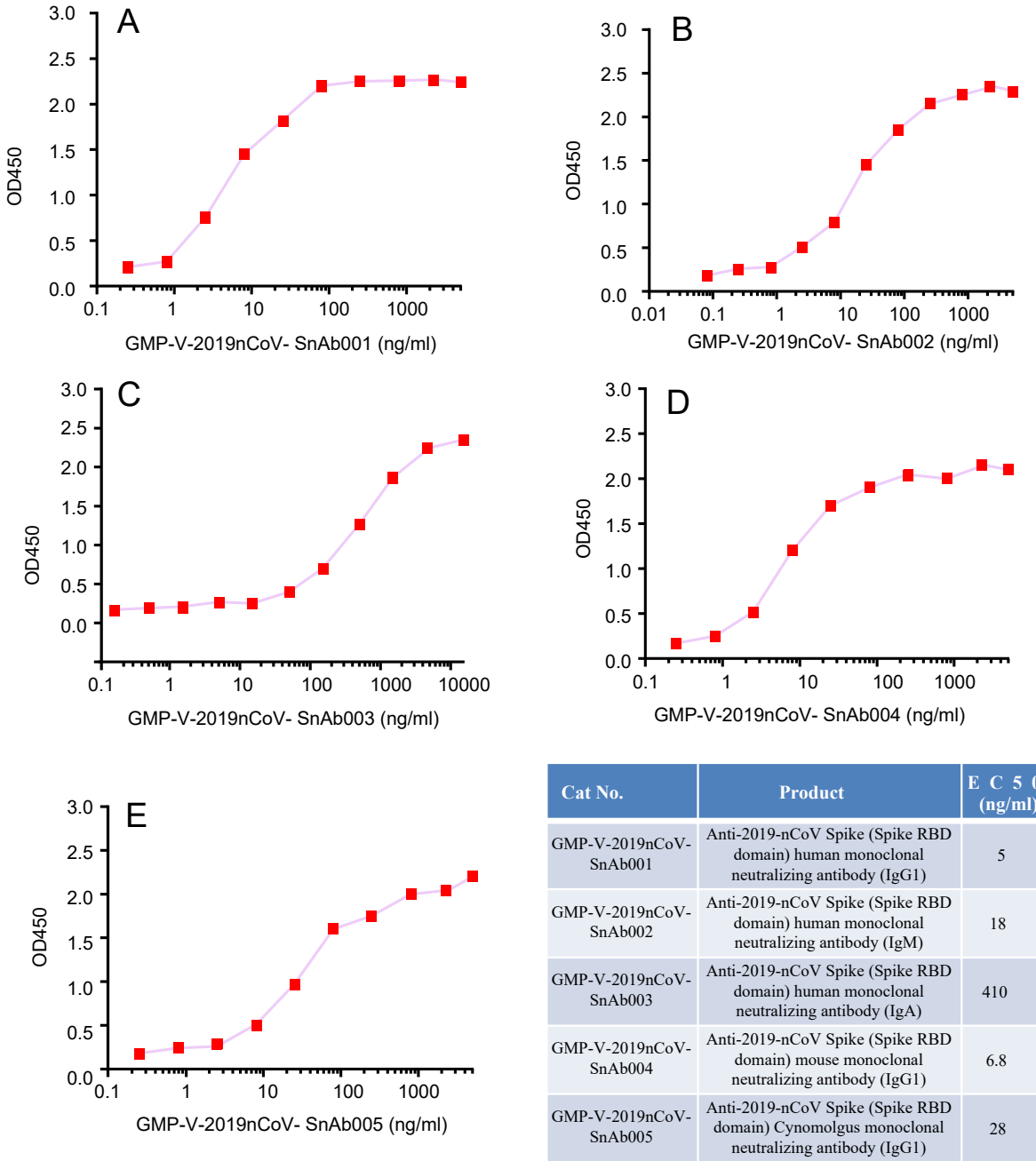


Figure. The Pseudovirus (PSV) Based Cell Entry assay was performed on 293T-hACE2 cells infected with GeneMedi-SARS-CoV-2 WT and Spike Mutation Variants (D614G, S943P, V367F, G476S, V483A, H49Y, Q239K, A831V, P1263L, D839Y/N/E:D839Y,D839N,D839E) Pseudovirus (PSV) Infection rate was determined by RFP+GFP+/GFP+ with FACS validation.

GeneMedi's anti-2019-nCoV Spike Neutralizing antibodies (Nabs) and Spike RBD protein binding validation

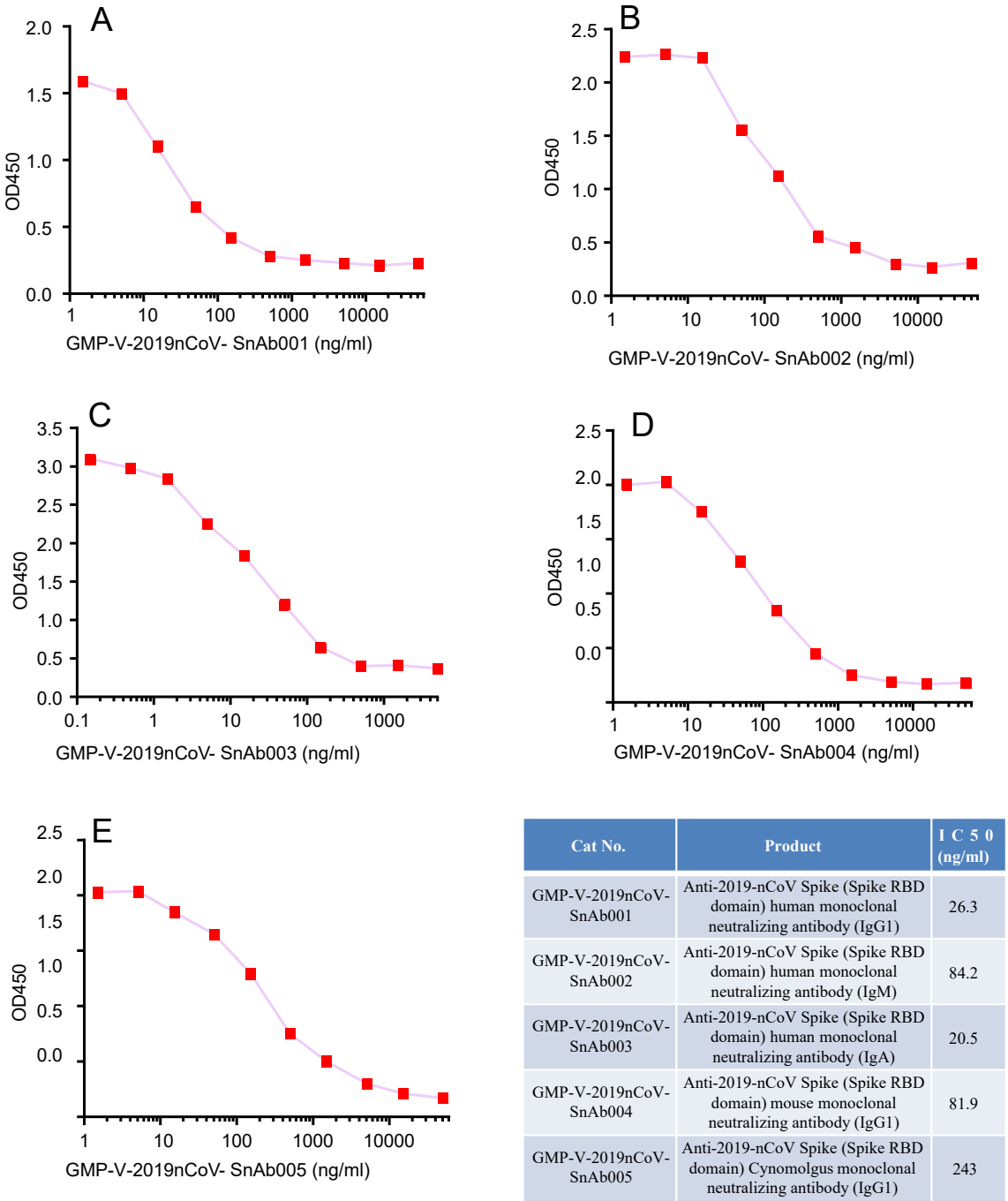


Cat No.	Product	EC50 (ng/ml)
GMP-V-2019nCoV-SnAb001	Anti-2019-nCoV Spike (Spike RBD domain) human monoclonal neutralizing antibody (IgG1)	5
GMP-V-2019nCoV-SnAb002	Anti-2019-nCoV Spike (Spike RBD domain) human monoclonal neutralizing antibody (IgM)	18
GMP-V-2019nCoV-SnAb003	Anti-2019-nCoV Spike (Spike RBD domain) human monoclonal neutralizing antibody (IgA)	410
GMP-V-2019nCoV-SnAb004	Anti-2019-nCoV Spike (Spike RBD domain) mouse monoclonal neutralizing antibody (IgG1)	6.8
GMP-V-2019nCoV-SnAb005	Anti-2019-nCoV Spike (Spike RBD domain) Cynomolgus monoclonal neutralizing antibody (IgG1)	28

Figure. The binding of GeneMedi's anti-2019-nCoV Spike Neutralizing antibodies (Nabs) to Recombinant 2019-nCoV(SARS-CoV-2) Spike RBD protein (GMP-V-2019nCoV-SRBD001) at 5.0ug/ml (100uL/well) was measured by ELISA.

A. GMP-V-2019nCoV-SnAb001:Anti-2019-nCoV Spike (Spike RBD domain) human monoclonal neutralizing antibody (IgG1)
B. GMP-V-2019nCoV-SnAb002:Anti-2019-nCoV Spike (Spike RBD domain) human monoclonal neutralizing antibody (IgM)
C. GMP-V-2019nCoV-SnAb003:Anti-2019-nCoV Spike (Spike RBD domain) human monoclonal neutralizing antibody (IgA)
D. GMP-V-2019nCoV-SnAb004:Anti-2019-nCoV Spike (Spike RBD domain) mouse monoclonal neutralizing antibody (IgG1)
E. GMP-V-2019nCoV-SnAb005:Anti-2019-nCoV Spike (Spike RBD domain) Cynomolgus monoclonal neutralizing antibody (IgG1)

GeneMedi's anti-2019-nCoV Spike Neutralizing antibodies (Nabs) competitive binding assay validation

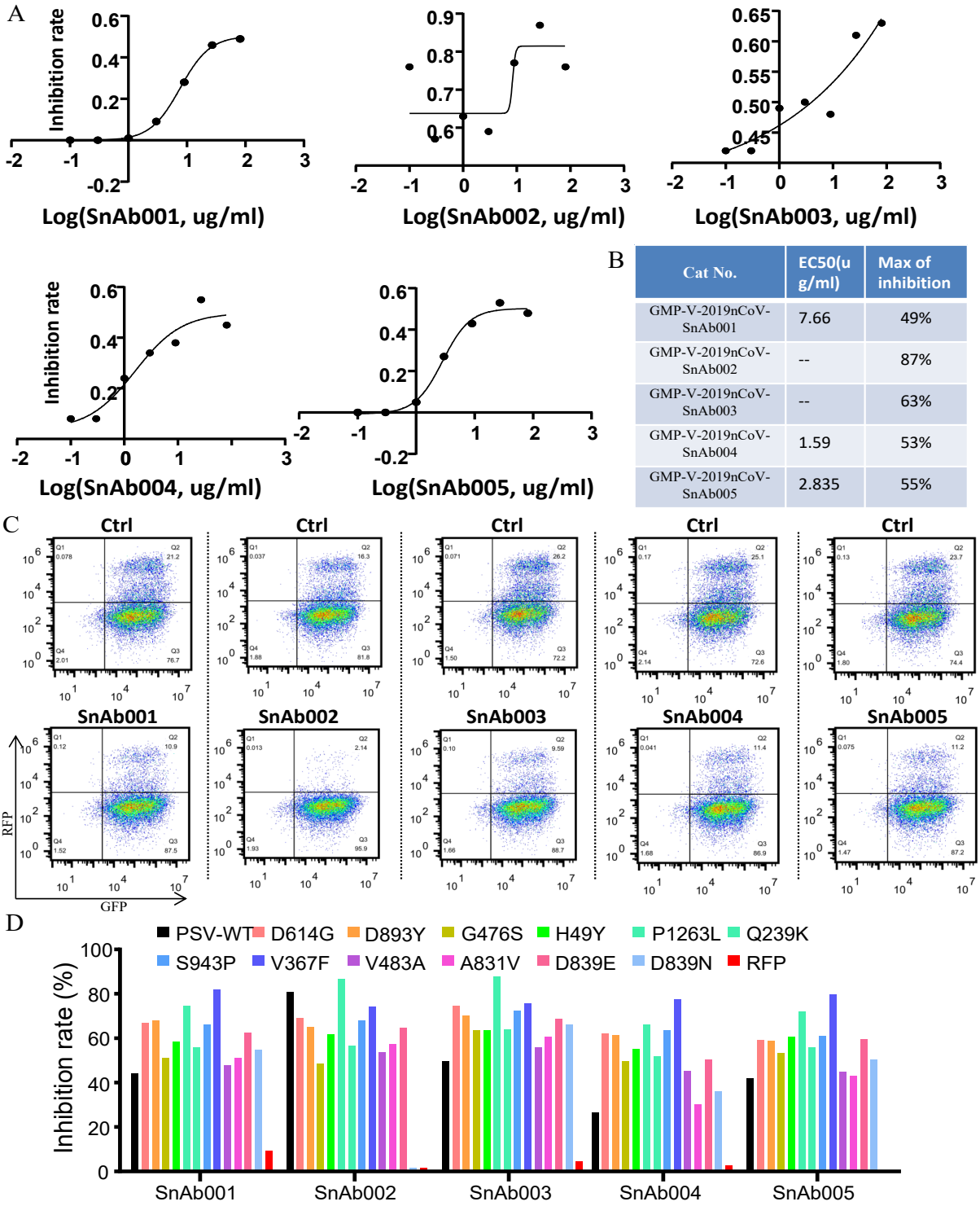


Cat No.	Product	IC50 (ng/ml)
GMP-V-2019nCoV-SnAb001	Anti-2019-nCoV Spike (Spike RBD domain) human monoclonal neutralizing antibody (IgG1)	26.3
GMP-V-2019nCoV-SnAb002	Anti-2019-nCoV Spike (Spike RBD domain) human monoclonal neutralizing antibody (IgM)	84.2
GMP-V-2019nCoV-SnAb003	Anti-2019-nCoV Spike (Spike RBD domain) human monoclonal neutralizing antibody (IgA)	20.5
GMP-V-2019nCoV-SnAb004	Anti-2019-nCoV Spike (Spike RBD domain) mouse monoclonal neutralizing antibody (IgG1)	81.9
GMP-V-2019nCoV-SnAb005	Anti-2019-nCoV Spike (Spike RBD domain) Cynomolgus monoclonal neutralizing antibody (IgG1)	243

Figure. GeneMedi's anti-2019-nCoV Spike Neutralizing antibodies (Nabs) block Recombinant 2019-nCoV(SARS-CoV-2) Spike RBD protein (GMP-V-2019nCoV-SRBD001) and hACE2 (GMP-H-ACE2002) binding.

A. GMP-V-2019nCoV-SnAb001:Anti-2019-nCoV Spike (Spike RBD domain) human monoclonal neutralizing antibody (IgG1)
B. GMP-V-2019nCoV-SnAb002:Anti-2019-nCoV Spike (Spike RBD domain) human monoclonal neutralizing antibody (IgM)
C. GMP-V-2019nCoV-SnAb003:Anti-2019-nCoV Spike (Spike RBD domain) human monoclonal neutralizing antibody (IgA)
D. GMP-V-2019nCoV-SnAb004:Anti-2019-nCoV Spike (Spike RBD domain) mouse monoclonal neutralizing antibody (IgG1)
E. GMP-V-2019nCoV-SnAb005:Anti-2019-nCoV Spike (Spike RBD domain) Cynomolgus monoclonal neutralizing antibody (IgG1)

GeneMedi-SARS-CoV-2 WT and Spike Mutation Variants Pseudovirus (PSV) Based Neutralizing Assay with GeneMedi's anti-2019-nCoV Spike Neutralizing antibodies (Nabs)



Cat No.	EC50(ug/ml)	Max of inhibition
GMP-V-2019nCoV-SnAb001	7.66	49%
GMP-V-2019nCoV-SnAb002	--	87%
GMP-V-2019nCoV-SnAb003	--	63%
GMP-V-2019nCoV-SnAb004	1.59	53%
GMP-V-2019nCoV-SnAb005	2.835	55%

Figure. The Pseudovirus (PSV) Based Neutralizing Assay was performed on 293T-hACE2 cells infected with GeneMedi-SARS-CoV-2 WT and Spike Mutation Variants (D614G, S943P, V367F, G476S, V483A, H49Y, Q239K, A831V, P1263L, D839Y/N/E:D839Y,D839N,D839E) Pseudovirus (PSV) under treatment of GeneMedi's anti-2019-nCoV Spike Neutralizing antibodies (Nabs) . Inhibition rate was determined by comparing the relative RFP+GFP+/GFP+ rate.

References

1 XiaolongCai. An Insight of comparison between COVID-19 (2019-nCoV) and SARS-CoV in pathology and pathogenesis. *Preprint*, doi:10.31219/osf.io/hw34x (2020).

2 Jean K. Millet1, Tiffany Tang3, Lakshmi Nathan3, Javier A. Jaimes4, Hung-Lun Hsu3,5, & Susan Daniel3, G. R. W. Production of Pseudotyped Particles to Study Highly Pathogenic Coronaviruses in a Biosafety Level 2 Setting. *J Vis Exp*, doi:10.3791/59010 (2019).

3 Nie, J. *et al.* Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. *Emerg Microbes Infect* **9**, 680-686, doi:10.1080/22221751.2020.1743767 (2020).

4 Jiang, S., Hillyer, C. & Du, L. Neutralizing Antibodies against SARS-CoV-2 and Other Human Coronaviruses. *Trends Immunol*, doi:10.1016/j.it.2020.03.007 (2020).

5 Zhang, G., Pomplun, S., Loftis, A. R., Loas, A. & Pentelute, B. L. The first-in-class peptide binder to the SARS-CoV-2 spike protein. doi:10.1101/2020.03.19.999318 (2020).

6 Xia, S. *et al.* Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. *Cell Res* **30**, 343-355, doi:10.1038/s41422-020-0305-x (2020).

7 Monteil, V. *et al.* Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. *Cell*, doi:10.1016/j.cell.2020.04.004 (2020).

8 Lei, C. *et al.* Neutralization of SARS-CoV-2 spike pseudotyped virus by recombinant ACE2-Ig. *Nat Commun* **11**, 2070, doi:10.1038/s41467-020-16048-4 (2020).

9 HuajunBai, X., XiaolongCai. Vaccines And Advanced Vaccines: -A landscape for advanced vaccine technology against infectious disease ,COVID-19 and tumor. *Preprint*, doi:10.31219/osf.io/ypgx4 (2020).

10 Hoffmann, M. *et al.* The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. *bioRxiv*, doi:10.1101/2020.01.31.929042 (2020).