

SARS-CoV-2 Pseudoviral Particles, Wuhan-Hu-1

CATALOG NUMBER: SCV2-PsV-0011, 1 mL; SCV2-PsV-001, 5mL

Description

It has been known that the coronaviruses SARS-CoV-2 and SARS-CoV use human ACE2 as the entry receptor and human proteases as the entry activators. The virus surface spike (S) protein mediates SARS-CoV-2 entry into cells. To fulfill its function, SARS-CoV-2 spike binds to the human ACE2 (hACE2) receptor through its receptor-binding domain (RBD) and is proteolytically activated by human proteases.

Our SARS-CoV-2 Pseudoviral Particles are replication-deficient MLV pseudotyped with the SARS-CoV-2 spike protein (Genbank Accession # YP_009724390.1) of the original SARS-CoV-2 strain (the Wuhan-Hu-1 isolate). They also contain the ORF for firefly luciferase as a reporter. They establish a pseudovirus entry assay for SARS-CoV-2 as the spike protein mediated cell entry can be conveniently measured via the luciferase reporter activity. This pseudovirus assay isolates the SARS-CoV-2 viral entry from other steps of the viral infection cycle. This pseudovirus is also referred to as SARS-CoV-2 614D pseudovirus.

A related item, Catalog # SCV2-PsV-614G, is the SARS-CoV-2 virus pseudotyped with the 614G variant spike protein.

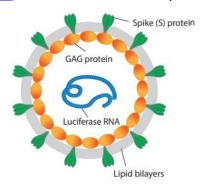


Figure 1. Illustration of the replication-deficient MLV particle pseudotyped with SARS-CoV-2 Spike protein

Reference: Identifying SARS-CoV-2 entry inhibitors through drug repurposing screens of SARS-S and MERS-S pseudotyped particles. https://pubs.acs.org/doi/pdf/10.1021/acsptsci.0c00112

Applications

Our Pseudovirus Particles (PP) generate robust chemiluminescent signals in cell assays when coupled with our firefly luciferase assay kit (Catalog # <u>CA-L165</u>), useful for 1) screening potential inhibitor to block SARS-CoV-2 entry and viral protein translation; 2) measuring the activity of and screening for neutralizing antibody against SARS-CoV-2 (refer to the Neutralization Assay Application Note).

Features

Robust: Excellent signal to noise (basal) ratio

Easy to use: Amenable to HTS format (96-well, 384-well and 1536-well format)

Contents

5ml; PP per mL > 1.0E+07

Storage

Upon receiving this item, store at -70 °C right away. Thaw* before immediate use.

*Note: read the instruction for thawing in the following protocol carefully. Do not aliquot and refreeze.

Shelf Life:

Six months from the date of shipping when stored at -70 °C



Please consider the environment before printing.



ASSAY PROTOCOL

Note: 1) require a luciferase assay kit (Catalog # CA-L165); 2) please read the protocol carefully prior to the experiment.

Cell Infection:

- Count Vero E6 cells or HEK293-ACE2 cells (Catalog # CL-hACE2-002) to be infected and seed ~20K cells per well into appropriate 96-well plates (50 µl per well) DMEM with 10% HyClone™ FetalClone™ II Serum (no antibiotics) or 5K cells per well into appropriate 384-well plates (15 μl per well).
- 2. Culture cells overnight to make sure the cells stably adhere to the plates.
- 3. On the 2nd day, remove media, add 50 µl SARS-CoV-2 pseudoviral particles* into each well (12.5 µl for 384-well plate). Spin at 700 rpm for 15 min at 4°C.

*Note: thaw the pseudoviral particles quickly in the room temperature water (< 30 minutes, do not shake) and use right away. Discard the unused portion (do not re-freeze or leave it on ice for later use).

- 4. Incubate for 2 hrs at 37 °C.
- 5. Add 50 μl DMEM with 10% FC into each well (12.5 μl for 384-well plates).
- 6. Incubate for 48 hrs at 37 °C.

Measurement of Luciferase Activity in Infected cells

- Do not remove medium. Add 100 µl eEnzyme's luciferase assay WORKING SOLUTION (25 µl for 384-well plate) directly into each well. Refer to the protocol of "Firefly Luciferase Assay Kit" (eEnzyme Cat.# CA-L165).
- 2. Read in a luminescence plate reader and record the data. (Note: the RLU values are higher from the 96-well.)

Data Analysis

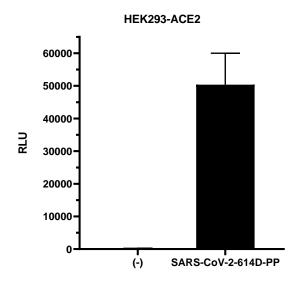


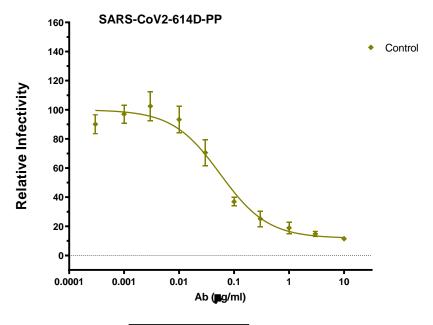
Figure 2. Pseudoviral Particle (PP) Infection Assays Pseudoviral particles on HEK293-ACE2 cells in 384-well format (BioTek Synergy II, 255 gain) SARS-CoV-2-614D-PP: MLV w/ SARS-CoV-2 Wuhan-Hu-1 spike protein (SCV2-PsV-001)

(-): MLV control (w/o spike protein) (Catalog # PsV-001)









	Control
Bottom	11.78
Тор	100.0
LogIC50	-1.253
IC50	0.05590
Span	88.24

Figure 3. SARS-CoV-2 Viral Infection Inhibiting Test by Neutralization Antibodies.

HEK293-ACE2 cells incubated with SARS-CoV-2 Pseudoviral Particles (Catalog # SCV2-PsV-0011) under various amount of neutralizing antibody.

Legend: SARS-CoV2-614D-PP, SARS-CoV-2 Pseudoviral Particles (Catalog # SCV2-PsV-001) "Control", neutralizing antibody used in this viral infection inhibition assay, SCV2-SA-11m.

Updated: Dec. 22, 2021