

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

CATALOG NUMBER: SCV2-PsV-0011

Size: 5 x 1mL

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.

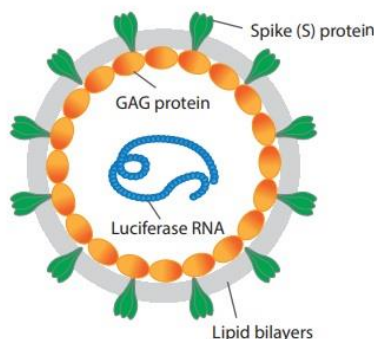


Figure 1. Illustration of the replication-deficient MLV particle pseudotyped with SARS-CoV-2 Spike protein

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 # [CA-L165](#)), 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

5 tubes of 1ml; 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

ASSAY PROTOCOL

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

Cell Infection:

1. 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

1. 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

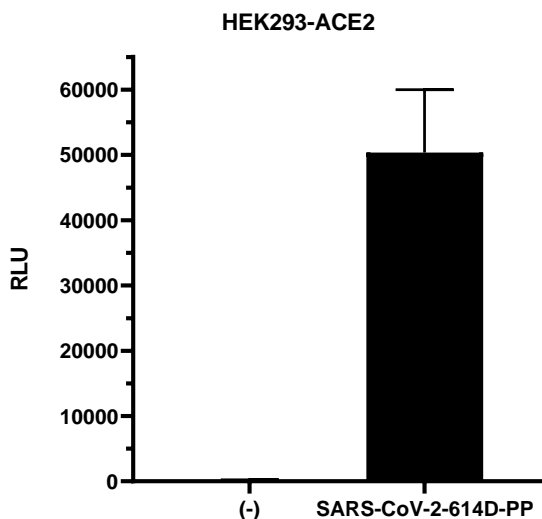
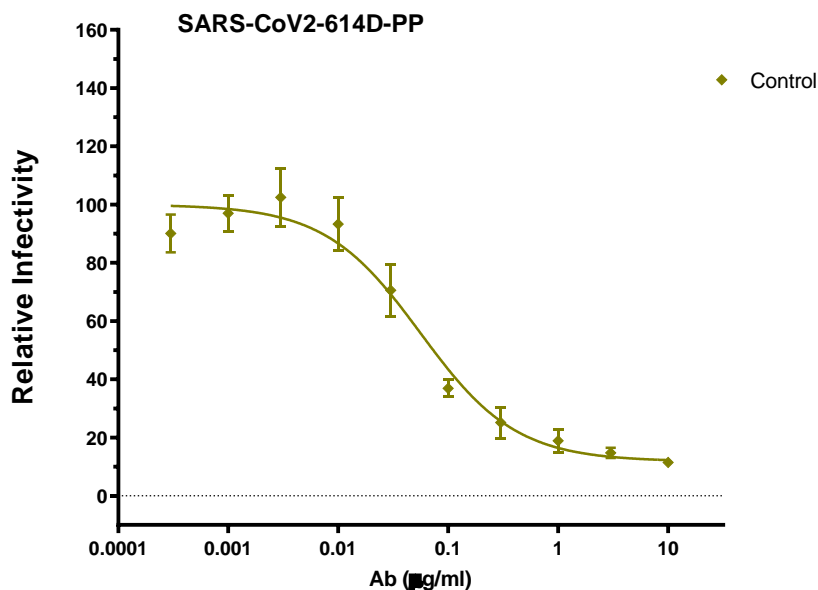


Figure 2. Pseudoviral Particle (PP) Infection Assays

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
Top	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](#).