

SARS-CoV-2 Pseudoviral Particles

CATALOG NUMBER: SCV2-PsV-001, 2x 5 mL

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 carrying the original D614 genotype (Genbank Accession # YP_009724390.1). 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.

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

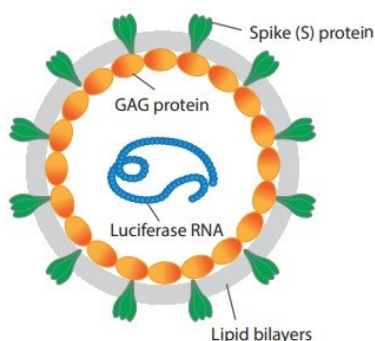


Figure 1. Illustration of the replication-deficient MLV particle pseudotyped with SARS-CoV-2 Spike protein

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 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

10 ml (2 tubes, 5 mL/tube), for 2 multi-well plates. Pseudoviral particles per mL $\geq 1.0E+08$

Storage

Upon receiving this item, store at -70°C right away. Thaw each vial immediately before use (**cannot be aliquoted and refreeze**).

Shelf Life:

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

ASSAY PROTOCOL

Note: requires a luciferase assay reagent (Catalog # [CA-L165](#))

Cell Infection:

1. Count Vero E6 cells or HEK293-ACE2 cells (Catalog # [CL-hACE2-001](#)) to be infected and seed ~20K cells per well into 96-well plates (50 µl per well) DMEM with 10% HyClone™ FetalClone™ II Serum (no antibiotics) or 5K cells per well into 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 immediately before use (take about 1 hour to thaw in ice) and use it within 2 hours.

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. Remove supernatant.
2. Add 100 µl eEnzyme's luciferase assay reagent (20 µl for 384-well plates).
3. Read in a luminescence plate reader and record the data. (Note: the RLU values are higher from the 96-well.)

Data Analysis

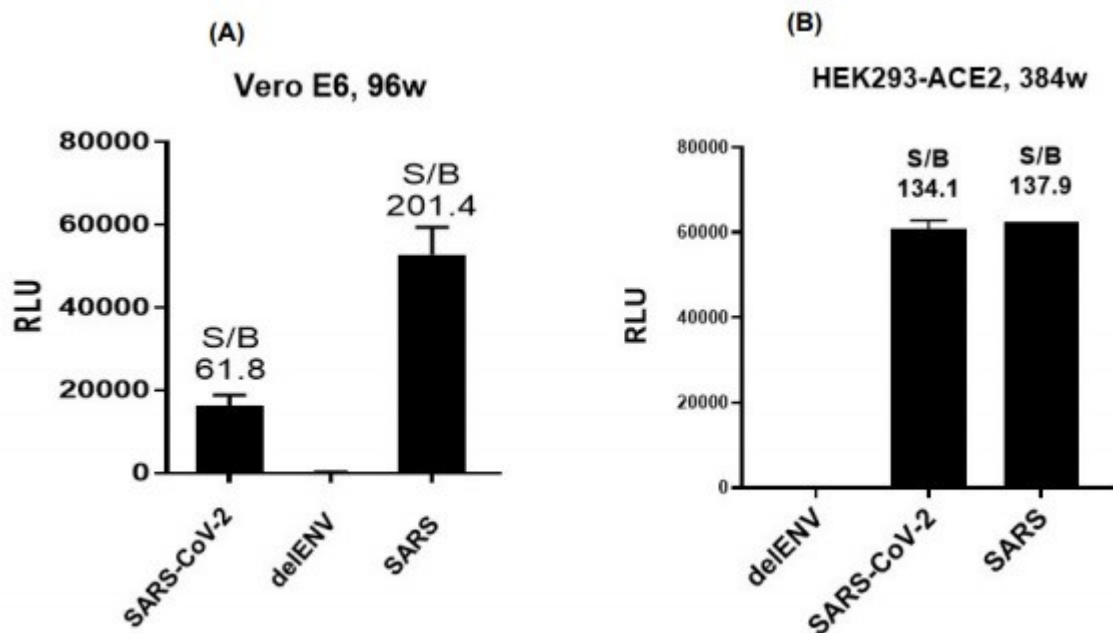


Figure 2. Pseudoviral Particle (PP) Infection Assays

(A) SARS and SARS-CoV-2 pseudoviral particles on Vero E6 cells in 96-well format

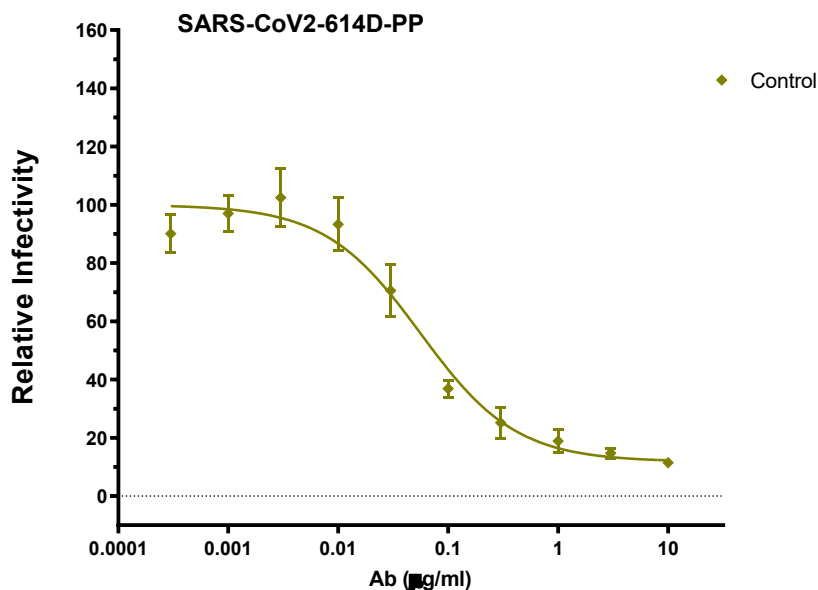
(B) SARS and SARS-CoV-2 pseudoviral particles on HEK293-ACE2 cells in 384-well format

Legends: 1) **SARS-CoV-2:** MLV w/ SARS-CoV-2 spike protein (SCV2-PsV-001)

delENV: MLV control (w/o envelope spike protein) (Catalog # [PsV-001](#))

SARS: MLV w/ SARS-CoV spike protein

2) **S/B:** Signal RLU of PP w spike protein / Baseline RLU of pp w/o spike protein



	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-001) 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](#).

