

SARS-CoV-2 Pseudoviral Particles, Indian B.1.617 Variant

CATALOG NUMBER: SCV2-PsV-IN, 5 mL

V.230201

Description

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

Our SARS-CoV-2-IN Pseudoviral Particles are replication-deficient MLV pseudotyped with the SARS-CoV-2 spike protein of the Indian variant B.1.617, also known as G/452R.V3, (GISAID sequence accession number EPI_ISL_1547802). They also contain the ORF for firefly luciferase as a reporter. They establish a pseudovirus cell entry assay mediated by the SARS-CoV-2 spike protein that can be conveniently measured via luciferase reporter activity. This pseudovirus assay isolates the SARS-CoV-2 viral entry from other steps of the viral infection cycle.

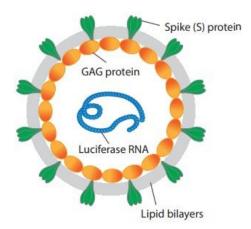


Figure 1. Illustration of the replication-deficient MLV particle pseudotyped with SARS-CoV-2 Spike protein

Applications

Our Pseudovirus Particles 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-IN (refer to <u>the Neutralization Assay Application Note</u>).

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 ml, for one multi-well plate; PP per mL > 1.0E+07

Storage

Upon receiving it, 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 store at -70 °C





ASSAY PROTOCOL

Note: require a luciferase assay reagent (Catalog # CA-L165).

Cell Infection:

- 1. Count HEK293-ACE2 cells (Catalog # <u>CL-hACE2-002</u>) 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-IN 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 42 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.

Data Analysis

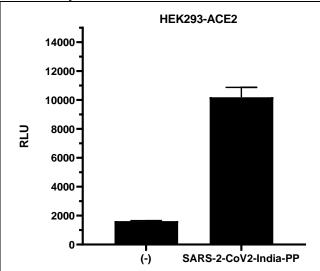


Figure 2. Pseudoviral Particle (PP) Infection Assays SARS-CoV-2-IN variant pseudoviral particles on HEK293-ACE2 cells in 384-well format. (Luminescence plate reader: PerkinElmer WALLAC VICTOR² 1420 Multilabel Counter)

Legends:

SARS-2-CoV-India-PP: SARS-CoV-2 Indian B.1.617 variant MLV Pseudovirus Particles (SCV2-PsV-IN)
(-): MLV control (w/o envelope spike protein) (Catalog # PsV-001)

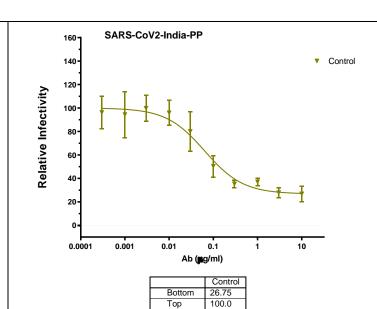


Figure 3. SARS-CoV-2 Viral Infection Inhibiting Test by Neutralization Antibodies.

LogIC50

Span

-1.196

73.25

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

Legend: SARS-C0V2-India-PP, SARS-CoV-2 India variant Pseudoviral Particles (Catalog # SCV2-PsV-IN) "**Control**", neutralizing antibody used in this viral infection inhibition assay, <u>SCV2-SA-11m</u>.