

## SARS-CoV-2 Pseudoviral Particles

### 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 Pseudoviral Particles** are replication-deficient MLV pseudotyped with the SARS-CoV-2 spike protein of the SARS-CoV-2 variant. 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.

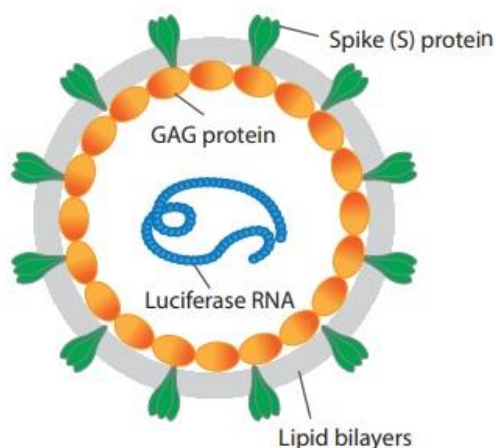


Figure 1. Illustration of the replication-deficient MLV particle pseudotyped with SARS-CoV-2 Spike protein

### Applications

Our Pseudovirus Particles (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 variant (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 ml, for one multi-well plates; 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 stored at -70 °C

## ASSAY PROTOCOL

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

### Cell Infection:

1. Count 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 2<sup>nd</sup> 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).**

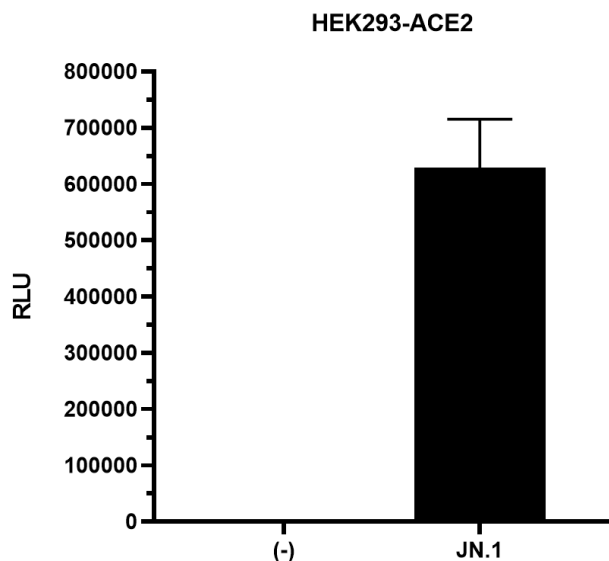
4. Incubate for 2 hrs at 37 °C.
5. Add 50 µl DMEM with 10% FC into each well (12.5 µl for 384-well plates).
6. Incubate for 42 hrs at 37 °C.

### Measurement of Luciferase Activity in Infected cells

1. Do not remove supernatant. Add 100 µl eEnzyme's luciferase assay WORKING SOLUTION (25 µl for 384-well plates). 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 Example

The data from JN.1 pseudoviral particles is used here as an illustrative example. *It is important to note that the infectivity of pseudoviral particles derived from different SARS-CoV-2 strains and variants exhibits substantial variation. This observed diversity in measured RLU values can be attributed to the distinct mutational profiles of each strain.*



**Figure 2. Pseudoviral Particle (PP) Infection Assays (SARS-CoV-2 JN.1 subtype pseudoviral particles on HEK293-ACE2 cells)** (Luminescence plate reader: BioTek Synergy 2, gain 255)

**Legends:**

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

JN.1: SARS-CoV-2 JN.1 variant MLV pseudoviral particles (SCV2-PsV-JN.1)