

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

Cat. No. SARS-CoV-2-07PsV **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Our SARS-CoV-2 Pseudoviral Particles are replication-deficient MLV pseudotyped with the SARS-CoV-2 spike protein 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.
Species	SARS-CoV-2
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.
Usage	Note: 1) require a luciferase assay kit; 2) please read the protocol carefully prior to the experiment. Cell Infection: 1. Count Vero E6 cells or HEK293-ACE2 cells to be infected and seed ~20K cells per well into appropriate 96-well plates (50 µL per well) DMEM with 10% 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.

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3. On the 2nd day, remove media, add 50 μ L SARS-CoV-2 pseudoviral particles* into each well (12.5 μ L for 384-well plate). Spin at 700 rpm for 15 min at 4 centigrade.
 *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 centigrade.

5. Add 50 μ L DMEM with 10% FC into each well (12.5 μ L for 384-well plates).

6. Incubate for 48 hrs at 37 centigrade.

Measurement of Luciferase Activity in Infected cells

1. Do not remove medium. Add 100 μ L luciferase assay WORKING SOLUTION (25 μ L for 384-well plate) directly into each well.

2. Read in a luminescence plate reader and record the data. (Note: the RLU values are higher from the 96-well.)

Applications

Our Pseudovirus Particles (PP) generate robust chemiluminescent signals in cell assays when coupled with our firefly luciferase assay kit, 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.

Shelf Life

Six months from the date of shipping when stored at -70 centigrade.

Features

Robust: Excellent signal to noise (basal) ratio

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

Storage

At -70 centigrade.

Shipping

Shipped on dry ice.

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References

1. A novel linker-immunodominant site (LIS) vaccine targeting the SARS-CoV-2 spike protein protects against severe COVID-19 in Syrian hamsters. *Emerging Microbes & Infections*, May 2021.
2. Immunogenicity and Neutralizing Activity Comparison of SARS-CoV-2 Spike Full-Length and Subunit Domain Proteins in Young Adult and Old-Aged Mice. *Vaccines*, Feb. 2021.
3. Serum Antibody Profile of a Patient With Coronavirus Disease 2019 Reinfection. *Clinical Infectious Diseases*, Sept. 2020.

Pseudoviral Particle (PP) Infection Assays

Pseudoviral particles on HEK293-ACE2 cells in 384-well format.

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

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

HEK293-ACE2 cells incubated with SARS-CoV-2 Pseudoviral Particles under various amount of neutralizing antibody.

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