SARS-CoV-2 (Y453F, D614G) Pseudoviral Particles

CATALOG NUMBER: SCV2-PsV-453F, 2x 5 mL

Description

It has been known that SARS coronavirus 2 (SARS-CoV-2) uses 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 then proteolytically activated by host proteases.

Our SARS-CoV-2 Pseudoviral Particles are replication-deficient MLV pseudotyped with the SARS-CoV-2 spike protein. They also contain the ORF for firefly luciferase as a reporter. They establish a pseudovirus entry assay for SARS-CoV-2 as the S 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 new SARS-CoV-2 strain with an amino acid change at position 453 from Tyr to Phe besides the change at position 614 from Asp to Gly in the viral S protein predominated over time in locales where it was found. To support the study of this new SARS-CoV-2 strain, we established this **SARS-CoV-2-614G-Y453F Pseudoviral Particles** with the spike protein carrying the 453F and 614G genotype (Genbank Accession # YP_009724390.1, p.453 Y->F, p.614 D->G)

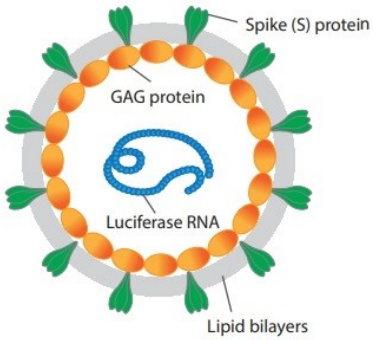

Figure 1. Illustration of the replication-deficient MLV particle pseudotyped with SARS-CoV-2 Spike protein

Applications

Our Pseudovirus Particles generate robust chemiluminescent signals in cell assays when coupled with our firefly luciferase assay kit (Catalog # 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-2-614G-453F (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

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 store at -70 °C

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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 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 remove medium. Add 100 µl eEnzyme's luciferase assay reagent (25 µl for 384-well plates).
2. Read in a luminescence plate reader, and record the data.

Data Analysis

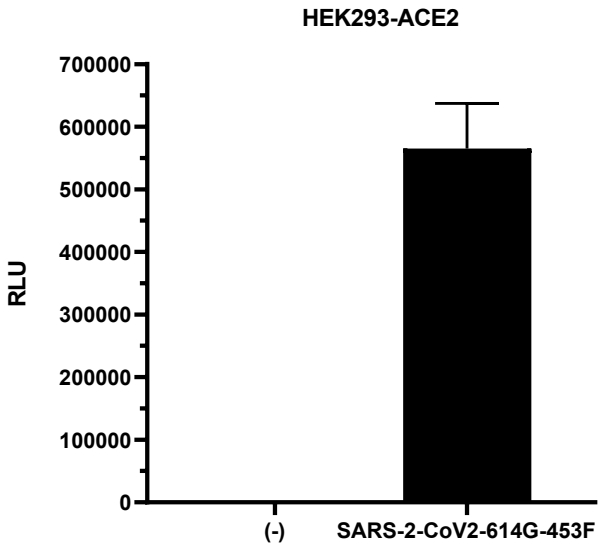

Figure 2. Pseudoviral Particle (PP) Infection Assays.

(-): the negative control (Catalog # PsV-001);

SARS-2-CoV2-614G-453F: the PP pseudotyped with the 453F 614G spike protein (Catalog # SCV2-PsV-453F).

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