MERS Pseudoviral Particles

CATALOG NUMBER: MERS-PsV-001, 5 mL

Description

Our MERS Pseudoviral Particles are replication-deficient MLV pseudotyped with the MERS spike protein (Genbank Accession # AHY21469.1). They also contain the ORF for firefly luciferase as a reporter. They establish a pseudovirus entry assay for MERS as the spike protein mediated cell entry can be conveniently measured via the luciferase reporter activity. This pseudovirus assay isolates the MERS viral entry from other steps of the viral infection cycle.

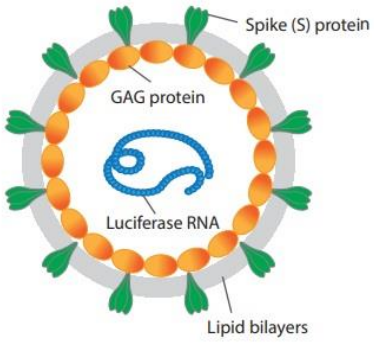

Figure 1. Illustration of the replication-deficient MLV particle pseudotyped with MERS Spike protein

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 MERS entry and viral protein translation; 2) measuring the activity of and screening for neutralizing antibody against MERS (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 plate

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 stored at -70 °C

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ASSAY PROTOCOL

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

Cell Infection:

1. Count Vero E6 cells or HUH-7 cells to be infected and seed ~20K cells per well into appropriate 96-well plates (50 µl per well) DMEM with 10% HyClone™ FetalClone™ II 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.
3. On the 2nd day, remove media, add 50 µl MERS 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. Remove the medium (this would result cleaner results. It is OK not to remove the medium in order to obtain higher RLU). 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. (Note: the RLU values are higher from the 96-well.)

Data Analysis

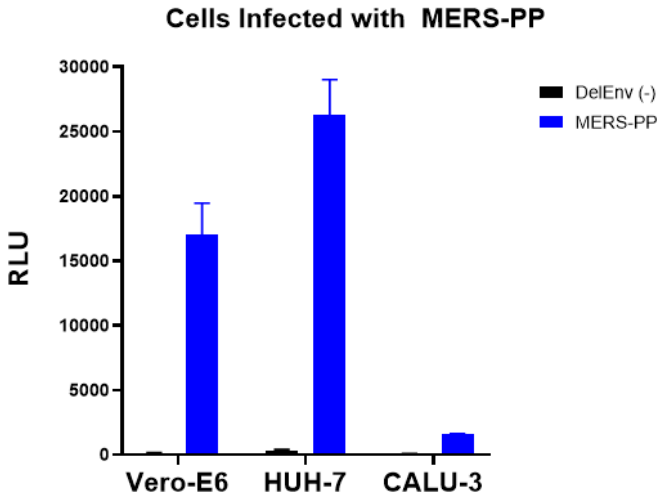

Figure 2. Pseudoviral Particle (PP) Infection Assays
MERS pseudoviral particles with Vero-E6 cells, or HUH-7 cells, or CALU-3 cells in 96-well format.
Legends: MERS-PP: MLV w/ MERS spike protein (MERS-PsV-001)
DelENV: MLV control (w/o envelope spike protein) (Catalog # PsV-001)

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