Influenza A (A/AW/SC/2021) H5N1 Pseudovirus, MLV-based

CATALOG NUMBER: MPsV-H5N1-SC21

Size: 1 mL, 5 mL

Description

This Influenza A H5N1 pseudovirus (or pseudoviral particle) is replication-deficient MIV (Murine Leukemia Virus) pseudotyped with the HA and NA proteins of the influenza A virus (A/American Wigeon/South Carolina/USDA-000345-001/2021)(H5N1) strain. They also contain the ORF for firefly luciferase (FLuc) as a reporter. Upon the Pseudoviral Particles (PP) infecting the cells, the reporters will be expressed in the transduced cells. By measuring the FLuc activity in the cells, the infectivity of the PP can be determined.

Gene/Protein Sources

HA (A/AW/SC/2021/H5N1) (GISAID # EPI2709137)
NA (A/AW/SC/2021/H5N1) (GISAID # EPI2709127)

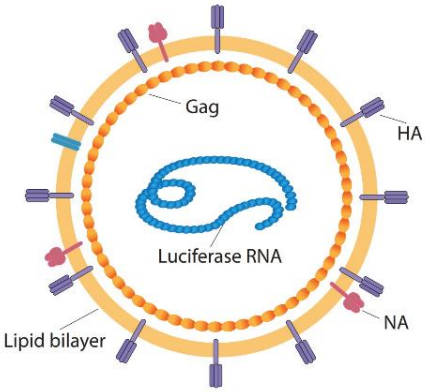

Figure 1. Illustration of the replication-deficient MLV particle pseudotyped with H5N1proteins

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 influenza A entry; 2) tittering neutralizing antibody against influenza A H5N1.

Contents

1 mL; 5mL (for one multiplate)

Storage

Upon receiving it, store at -70 °C right away. Thaw* before immediate use.
Note: Carefully read the thawing instructions in the following protocol. Avoid repeated freeze-thaw cycles.

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 293T cells 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 this pseudovirus* 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.
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. No need to remove supernatant. 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

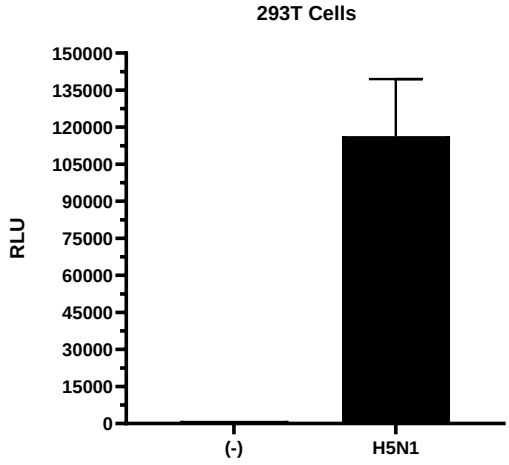

Figure 2. Pseudoviral Particle (PP) Infection Assays
(Pseudoviral particles on 293T cells in 96-well format; Luminescence plate reader: BioTek Synergy 2, gain 255)
H5N1: MLV-based pseudovirus w/ influenza A H5N1 proteins (MPsV-H5N1-SC21)
(-): Negative control (MLV-based pseudoviral particles, w/o H5N1 proteins)