Eastern Equine Encephalitis Virus (EEEV) Pseudoviral Particles

CATALOG NUMBER: PsLv-EEEV-01, 1x 5 mL

Description

This EEEV pseudovirus (also referred to as pseudotyped lentivirus) is replication-deficient lentiviral particle engineered to display the envelope glycoproteins of Eastern Equine Encephalitis Virus (EEEV). It carries dual reporter genes-firefly luciferase (FLuc) and GFP-enabling versatile detection. Upon infecting target cells, the Pseudoviral Particles (PP) mediate FLuc expression, allowing quantitative assessment of infectivity via luciferase activity. Additionally, GFP serves as a fluorescent marker for visual confirmation and downstream analysis of transduced cells.

Gene/Protein Sources

EEEV (GenBank accession No. MT782294)

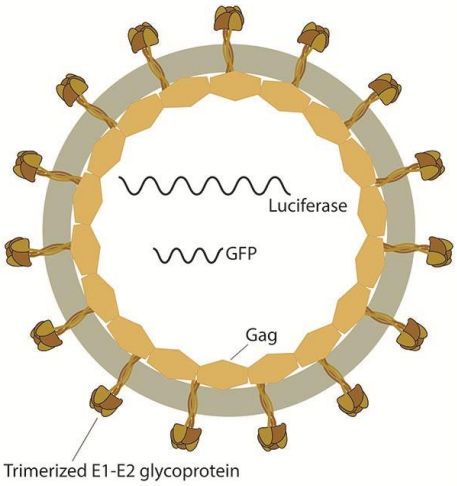

Figure 1. Illustration of the replication-deficient lentivirus pseudotyped with EEEV Spike E1/E2 glycoproteins

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 EEEV entry and viral protein translation; 2) as immunogens in preclinical vaccine studies; 3) virus-host interactions, such as cell entry and receptor binding studies; 4) measuring the activity (ELISA) of and screening for neutralizing antibody against EEEV (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/vail, for one multi-well plate (PP per mL >4.7E+07)

Storage

Upon receiving this item, store at -80 ºC 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 -80 ºC

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ASSAY PROTOCOL

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

Cell Infection:

1. Count VLDLR cells or HEK293 cells (Catalog # CL-VLDLR-01) 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 (12.5 µl per well).
2. Add 50 µL EEEV 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).
3. Incubate for 72 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 (BioTeck Synergy 2) and record the data. (Note: the RLU values are higher from the 96-well.)

Data Analysis

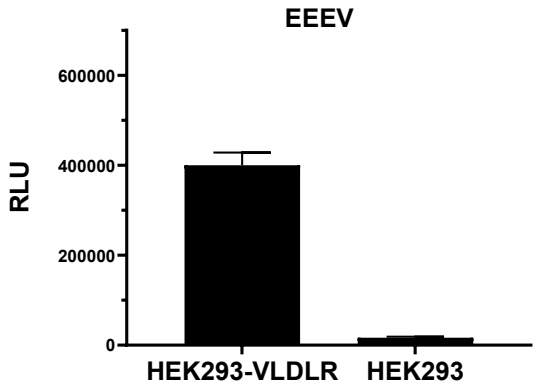

Figure 2. Pseudoviral Particle (PP) Infection Assays: HEK293 control cells and HEK293-VLDLR stable cells were infected with EEEV pseudoviral particles in a 384-well plate format.

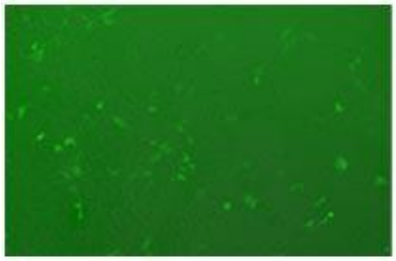

Figure 3. Transduction of Hek293 cells using EEEV pseudoviral particles: GFP-positive cells indicate successful transfection with EEEV PPs.

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