Ebola Pseudoviral Particles, Zaire Ebolavirus/Makona 2014

CATALOG NUMBER: EBOV-PsLv-MC14

Size: 1mL, 5 mL

Description

Ebola virus enters a cell by utilizing its surface glycoprotein (GP), which acts as a key to binding to specific receptors on the host cell membrane.

This **Ebola Pseudovirus** is replication-deficient Lentivirus (LV) pseudotyped with the surface glycoprotein of Zaire ebolavirus (*H.sapiens-wt*/GIN/2014/Makona-Coyah-955)(GenBank: AKG65250). The PseudoViral Particles contain the ORF for firefly luciferase as a reporter. These allow to establish an ebolavirus cell entry assay mediated by the Ebola virus glycoprotein which can be conveniently quantified via measuring luciferase activity in the cells. This pseudovirus assay isolates the Ebola viral entry from other steps of the viral infection cycle.

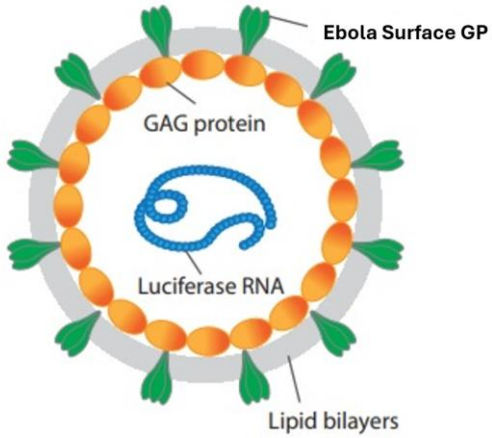

Figure 1. Illustration of the replication-deficient lentivirus particle pseudotyped with Ebola virus surface Glycoprotein (GP).

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

1 ml; OR 5 ml, for one multi-well plate. PP per mL > 1.0E+07

Storage

Upon receiving it, store at -70 ^{o}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 ^{o}C

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ASSAY PROTOCOL

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

Cell Infection

1. Count HEK293 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 Ebola 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 48-72 hrs at 37 °C.

Measurement of Luciferase Activity in Infected cells

1. Do not remove supernatant. Add 100 µl luciferase assay WORKING SOLUTION (25 µl for 384-well plates). Refer to the protocol of "Firefly Luciferase Assay Kit" (Cat.# CA-L165).
2. Read in a luminescence plate reader and record the data.

Data Analysis

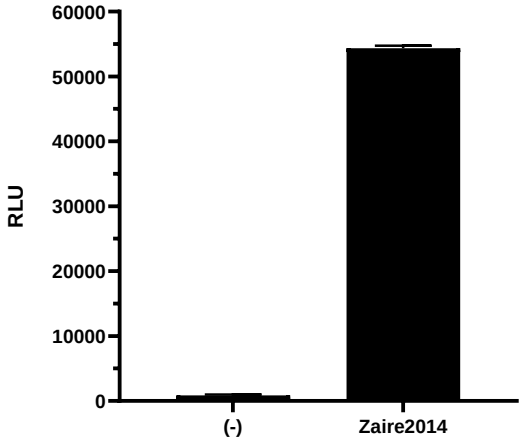

Figure 2. Pseudoviral Particle (PP) Infection Assays (Ebola pseudoviral particles on HEK293 cells)
(Luminescence plate reader: BioTek Synergy 2, gain 255)

Legends:
(-): lentivirus control (w/o envelope protein)
Zaire2014: Ebola pseudovirus particles (Catalog# EBOV-PsLv-MC14)

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