Data Sheet

Spike (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)

Catalog#: AMS.79981

Product Description

The pandemic coronavirus disease 2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). As the first step of the viral replication, the virus attaches to the host cell surface before entering the cell. The viral Spike protein recognizes and attaches to the Angiotensin-Converting Enzyme 2 (ACE2) receptor found on the surface of type I and II pneumocytes, endothelial cells, and ciliated bronchial epithelial cells. Drugs targeting the interaction between the Spike protein of SARS-CoV-2 and ACE2 may offer protection against the viral infection.

The SARS-CoV-2 Spike Pseudotyped Lentivirus were produced with SARS-CoV-2 Spike (Genbank Accession #QHD43416.1) as the envelope glycoproteins instead of the commonly used VSV-G. These pseudovirions also contain the enhanced GFP gene driven by a CMV promoter (Figure 1), therefore, the spike-mediated cell entry can be conveniently determined via eGFP activity. The SARS-CoV-2 Spike pseudotyped lentivirus can be used to measure the activity of neutralizing antibody against SARS-CoV-2 in a Biosafety Level 2 facility.

Application

1. Study the mechanism of viral transduction.
2. Screening for neutralizing antibodies for SARS-CoV-2 Spike and ACE2.

Formulation

The lentiviruses were produced from HEK293T cells in medium containing 90% DMEM + 10% FBS.

Titer

The titer will vary with each lot; the exact value is provided with each shipment.

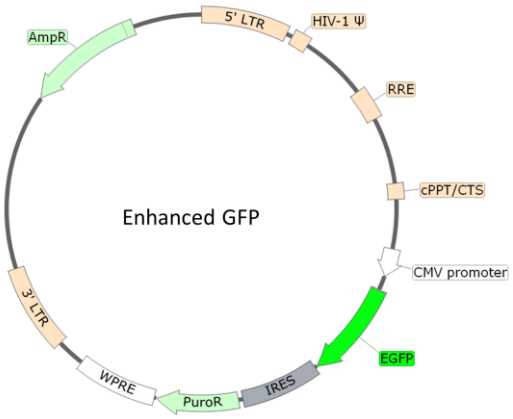

Figure 1. Schematic of the eGFP Reporter in SARS-CoV-2 Spike Pseudovirion

Storage
Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety
None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Materials Required but Not Supplied
- HEK293 growth medium or use
  Thaw Medium 1 (AMS.60187): MEM supplemented with 10% FBS, 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).
- ACE2-HEK293 Recombinant Cell Line (AMS.79951)
- Bald Lentiviral Pseudoviron (eGFP Reporter) (AMS.79987)

Assay Protocol
The following protocol is a general guideline for transducing ACE2-HEK293 cells using SARS-CoV-2 Spike pseudotyped lentivirus (eGFP reporter). The optimal transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements. In most cell types, the expression of the reporter gene can be measured approximately 48-72 hours after transduction.

1. Day 1: Harvest ACE2-HEK293 cells from culture and seed cells at a density of 5,000-10,000 cells per well into white opaque 96-well microplate in 50 µl of Thaw Medium 1 (AMS.60187). Incubate cells at 37°C with 5% CO_2 overnight.

   To demonstrate the transduction is dependent on ACE2, the same number of HEK293 parental cells are seeded in Thaw Medium 1 as control cells.

2. Day 2: Add 50 µl of SARS-CoV-2 Spike pseudotyped lentivirus (eGFP reporter) or bald lentiviral pseudovirion (eGFP reporter) into each well.

   Optional: Add polybrene to each well at a final concentration of 5 µg/ml.

   Alternatively, seeding cells and the transduction can be performed on the same day.

   Incubate the plates overnight at 37°C with 5% CO_2.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 50 µl of fresh Thaw Medium 1 to each well.
   If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 3. The target cells can be incubated with the virus for 48-72 hours before changing medium.

4. Day 4-5, approximately 48-72 hours after transduction, the expression of eGFP in the target cells was examined using fluorescence microscopy.

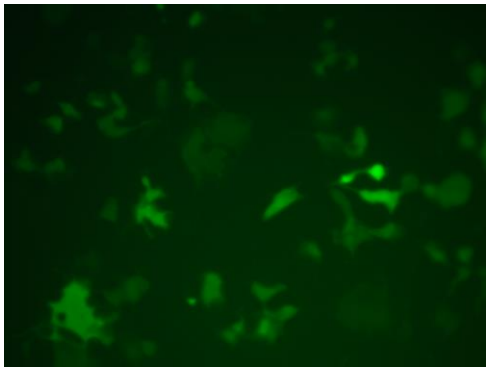

Figure 2. Transduction of ACE2-HEK293 cells using SARS-CoV-2 Spike pseudotyped lentivirus (eGFP reporter). Approximately 10,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 50 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (eGFP reporter) or bald lentiviral pseudovirion (eGFP reporter), AMS.79987. After18 hours of transduction, the medium was changed to fresh HEK293 growth medium (ThawMedium 1). After 66 hours of transduction, the expression of eGFP in the target cells wasobserved under a fluorescence microscope.

As negative controls, almost no eGFP expression was observed in ACE2-HEK cells transduced with Bald Lentiviral Pseudovirion (eGFP reporter) or HEK parental cells transduced with SARS-CoV-2-Spike pseudotyped lentivirus (eGFP reporter), indicating the transduction is dependent upon the ACE2 receptor.