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 and ACE2 may offer protection against the viral infection. A SARS-CoV-2 variant carrying the spike protein amino acid change D614G has become the most prevalent form in the global pandemic.

The SARS-CoV-2 Spike D614G Pseudotyped Lentivirus were produced with SARS-CoV-2 Spike (Genbank Accession #QHD43416.1; with D614G mutation) as the envelope glycoproteins instead of the commonly used VSV-G. These pseudovirions contain the firefly luciferase gene driven by a CMV promoter (Figure 1), therefore, the spike-mediated cell entry can be measured via luciferase activity. The SARS-CoV-2 Spike D614G pseudotyped lentivirus can be used to measure the activity of neutralizing antibody against SARS-CoV-2 in a Biosafety Level 2 facility.

Spike interacts with host cells by binding to membrane receptor ACE2 (angiotensin converting enzyme 2). Based on experiments performed by scientists at BPS Bioscience, we know that the wild-type SARS-CoV-2 spike pseudotyped lentiviruses transduce the following cells with great efficiency: ACE2-HEK293 cells (BPS Bioscience #79951), ACE2-CHO cells (BPS Bioscience #79959), ACE2-HeLa cells (BPS Bioscience #79958). They also efficiently transduce TMPRSS2-Vero E6 cells (BPS Bioscience #78081), which express high endogenous levels of ACE2 and were stably transfected with human serine protease TMPRSS2 required for the priming of Spike and fusion of the virion with the plasma membrane. By contrast, it has been shown by others that SARS-CoV-2 spike pseudotyped lentiviruses do not transduce parental Calu3 and Vero E6 cells very well [Neerukonda *et al*. 2021, PlosOne PMID: 33690649; Tandon *et al*. 2020, Scientific Reports PMID: 33154514; Condor Capcha *et al.* 2021, Front. Cardiovasc. Med. PMID: 33521067; Pisil *et al*. 2021, Pathogens PMID: 33540924].

SARS-CoV-2 variant pseudoviruses have been validated using ACE2-HEK293 cells but have not been tested in other cells.

As recommended in our protocol, 5 µl of virus/well in a 96-well plate provides a sufficient signal-to-noise ratio to perform inhibition studies. The amount of virus added to the cells can also be scaled down according to the user's need.

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.

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

These materials are not supplied with this lentivirus but are necessary to follow the designed protocol. BPS Bioscience media, reagents, and luciferase assay systems are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
ACE2-HEK293 Recombinant Cell Line	BPS Bioscience #79951
Anti-SARS-CoV-2 Spike neutralizing antibody	BPS Bioscience #100793
96-well tissue culture treated, white clear-bottom assay plate	Corning, #3610
ONE-Step™ luciferase assay system	BPS Bioscience #60690

Assay Protocol

The following protocol is a general guideline for transducing ACE2-HEK293 cells using SARS-CoV-2 Spike D614G pseudotyped lentivirus (Luciferase 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 (BPS Bioscience #60187). Incubate cells at 37°C with 5% CO_2 overnight.

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

2. Day 2: prepare serial dilutions of anti-Spike or anti-ACE2 antibody in Thaw Medium 1.

   To test anti-Spike antibody, preincubate 5 µl of the SARS-CoV-2 Spike D614G pseudotyped lentivirus with 5 µl of diluted anti-Spike antibody for 30 minutes. After incubation, add 10 µl of virus/antibody mix into each well of the ACE2-HEK293 cells.

   To test anti-ACE2 antibody, add 5 µl of diluted anti-ACE2 antibody into each well of ACE2-HEK293 cells and incubate for 30 minutes. At the end of the incubation, add 5 µl of SARS-CoV-2 Spike D614G pseudotyped lentivirus into each well.

For control wells, the same number of ACE2-HEK293 cells are seeded, but no virus or antibody are added.

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

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

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 50 µl of fresh Thaw Medium 1 to each well.

   If the tested antibody does not adversely affect the target cells, it is not necessary to change the medium on Day 3.

4. Day 4, approximately 48-60 hours after transduction, prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 50 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. The transduction efficacy is determined by measuring the luciferase activity.

Figures and Validation Data

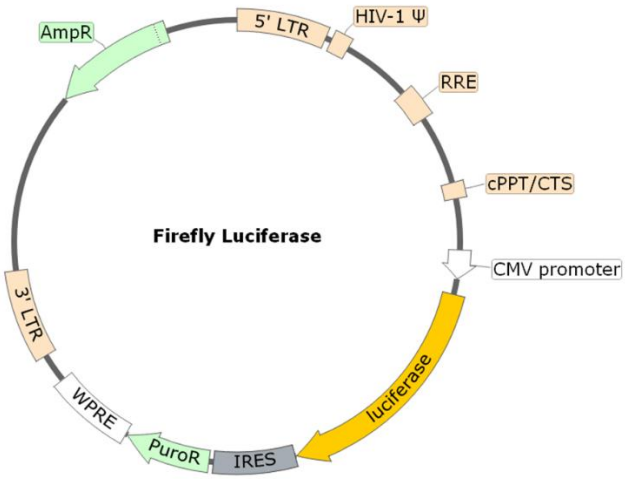

Figure 1. Schematic of the Luciferase Reporter in SARS-CoV-2 Spike Pseudovirion Storage
Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

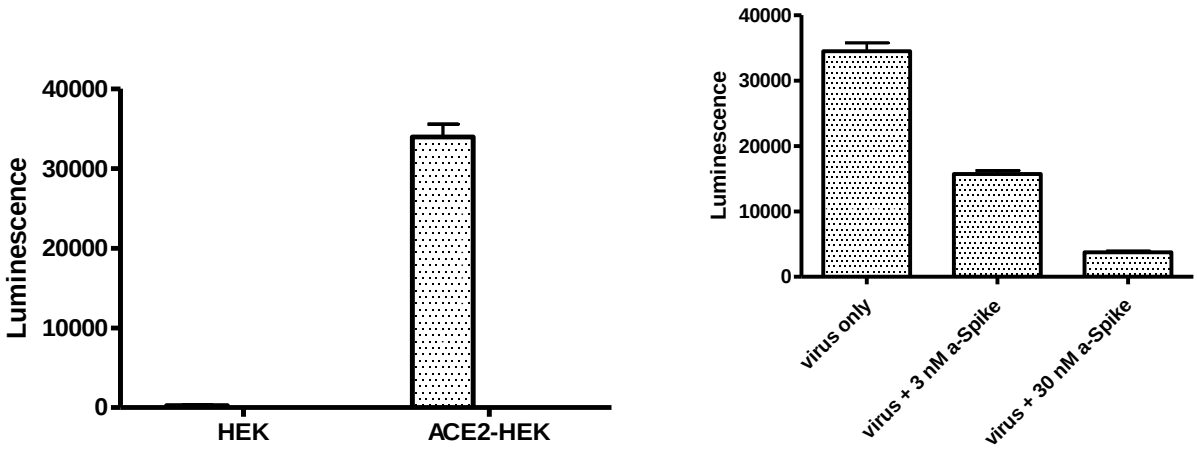

Figure 2. Transduction of ACE2-HEK293 Cells using SARS-CoV-2 Spike D614G Pseudotyped Lentivirus.
A. Approximately 10,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5 µl/well of SARS-CoV-2-Spike D614G pseudotyped lentivirus (Luc reporter) (BPS Bioscience #78028). After 18 hours of transduction, the medium was changed to fresh HEK growth medium (Thaw Medium 1). After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience, #60690) was added to cells to measure the luciferase activity. The SARS-CoV-2 Spike D614G pseudotyped lentivirus transduced ACE2-HEK293 cells with much greater efficiency compared with HEK293 parental cells, indicating the transduction is dependent upon ACE2 expression. *B.* Approximately 10,000 ACE2-HEK293 cells/well were transduced with 10 µl/well of SARS-CoV-2 Spike pseudotyped lentivirus (Luc reporter) mixed with anti-Spike antibody (BPS Bioscience #100793). After 18 hours of transduction, the medium was changed to fresh HEK growth medium (Thaw Medium 1). After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience #60690) was added to cells to measure the luciferase activity.

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Troubleshooting Guide
Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Spike Pseudotyped Lentivirus (Luciferase Reporter)	79942	500 µl x 2
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x 2
Spike Pseudotyped Lentivirus (eGFP Reporter)	79981	500 µl x 2
Bald Lentiviral Pseudoviron (eGFP Reporter)	79987	500 µl x 2
Spike Pseudotyped Lentivirus (Luciferase-eGFP Dual Reporter)	79982	500 µl x 2
Bald Lentiviral Pseudoviron (Luciferase-eGFP Dual Reporter)	79988	500 µl x 2
eGFP Lentivirus	79979	500 µl x 2
Firefly Luciferase-eGFP Lentivirus	79980	500 µl x 2
Negative Control Lentivirus	79578	500 µl x 2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x 2
Firefly Luciferase (Fluc) Lentivirus	79692	500 µl x 2