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 firefly luciferase gene driven by a CMV promoter (Figure 1), therefore, the spike-mediated cell entry can be conveniently measured via luciferase reporter 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. Supplied 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 or HEK293 Growth Medium	BPS Bioscience #60187
ACE2-HEK293 Recombinant Cell Line	BPS Bioscience #79951
Bald Lentiviral Pseudovirion (Luciferase Reporter)	BPS Bioscience #79943
Spike S1 Neutralizing Antibody (SARS-CoV-2) (Clone: 414-1)	BPS Bioscience #100793
Spike S1 Neutralizing Antibody (SARS-CoV-2) (Clone: 414-2)	BPS Bioscience #100792
Anti-ACE2 Antibody	R&D Systems #AF933
Recombinant ACE2 protein	BPS Bioscience #11003
96-well tissue culture treated, white clear-bottom assay plate	Corning #3610
ONE-Step™ luciferase assay system	BPS Bioscience #60690

For the transduction assay protocol below, Thaw Medium 1 (BPS Bioscience #60187) is utilized to thaw and maintain the ACE2 HEK293 cells. Depending upon the type of cell line used in conjunction with this lentivirus, the media will need to be changed and optimized for the cell type and customized protocol.

Media Formulation

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Thermo Fisher, Cat. #26140079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

Assay Protocol

The following protocol is a general guideline for transducing ACE2-HEK293 cells using SARS-CoV-2 Spike 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.

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 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 pseudotyped lentivirus into each well.

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

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

Alternatively, seeding cells and the transduction can be performed at 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 was determined by measuring the luciferase activity.

License Disclosure
Visit bpsbioscience.com/license for the label license and other key information about this product.

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

Figures and Validation Data

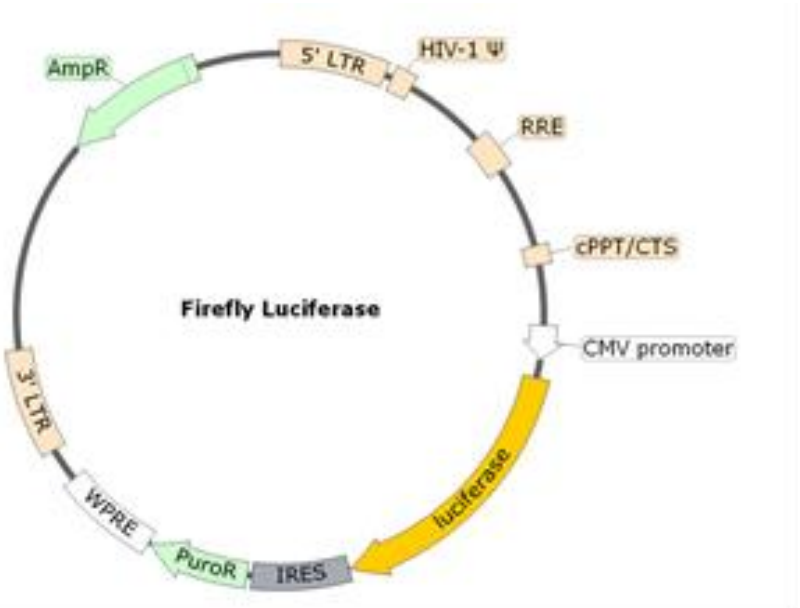

Figure 1. Schematic of the Luciferase Reporter in SARS-CoV-2 Spike Pseudovirion

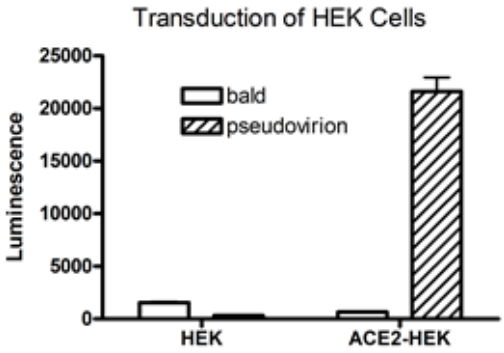

Figure 2. Transduction of ACE2-HEK293 cells
Approximately 10,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luc reporter). After 18 hours of transduction, the medium was changed to fresh HEK growth medium (Thaw Medium 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 pseudotyped lentivirus transduced ACE2-HEK293 with much greater efficiency compared with HEK293 parental cells, indicating the transduction is dependent upon ACE2 expression. The bald lentiviral pseudovirion (BPS Bioscience #79943), where no envelope glycoprotein is expressed, was used as a negative control.

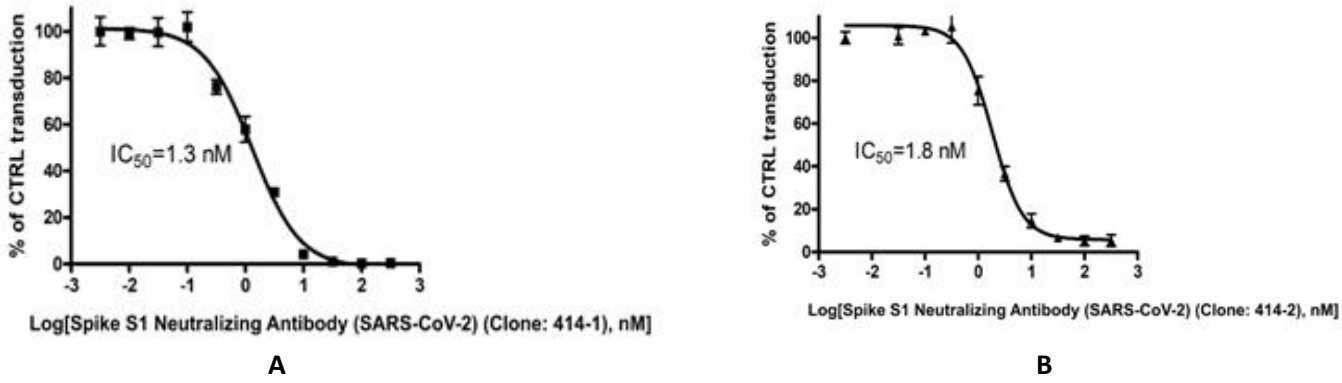

Figure 3: Neutralization assay by anti-SARS-CoV-2 Spike antibody
Approximately 10,000 ACE2-HEK293 cells/well were transduced with 10 µl/well of SARS-CoV-2 Spike pseudotyped lentivirus (Luc reporter)/anti-Spike antibody mix. After 18 hours of transduction, the medium was changed to fresh HEK growth medium (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 transduction efficiency was determined by measuring the luciferase activity. The transduction efficiency of the wells with virus only (no antibody treatment) was set as 100%, while the transduction efficiency of the wells without virus was set as 0%. The titration curves for Spike S1 Neutralizing Antibody (SARS-CoV-2) (Clone: 414-1) (BPS Bioscience #100793) and Spike S1 Neutralizing Antibody (SARS-CoV-2) (Clone: 414-2) (BPS Bioscience #100792) are shown in A and B respectively.

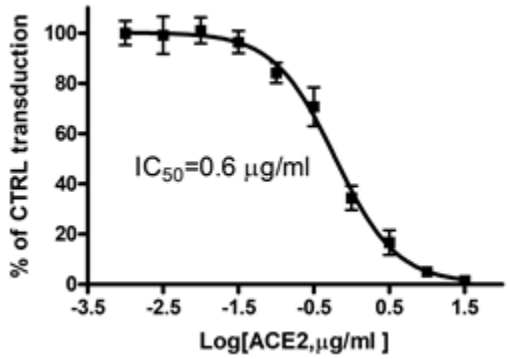

Figure 4: Neutralization assay by recombinant ACE2.
Approximately 10,000 ACE2-HEK293 cells/well were transduced with 10 µl/well mixture of SARS-CoV-2 Spike pseudotyped lentivirus (Luc reporter)/ACE2 (BPS Bioscience, #11003). After 18 hours of transduction, the medium was changed to fresh HEK growth medium (Thaw Medium 1). After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience #60690) was added to cells to measure the luciferase activity.

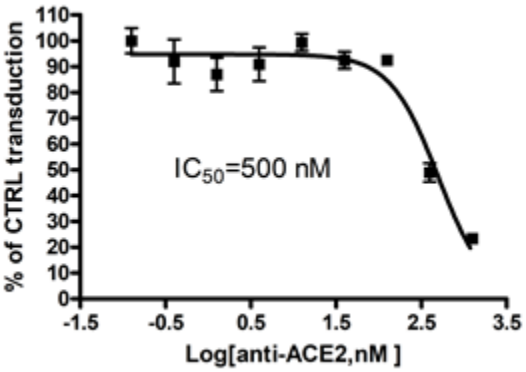

Figure 5: Neutralization assay by anti-ACE2 Antibody
Approximately 10,000 ACE2-HEK293 cells/well were preincubated with anti-ACE2 antibody (R&D Systems #AF933) for 30 minutes, and then transduced with 5 µl/well SARS-CoV-2 Spike pseudotyped lentivirus (Luc reporter). After 18 hours of transduction, the 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.

Related Products

Products	*Catalog #*	*Size*
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x 2
NFκB Luciferase Reporter Lentivirus)	79564	500 µl x 2
CRE Luciferase Reporter Lentivirus	79580	500 µl x 2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x 2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x 2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x 2
TCF/LEF Luciferase Reporter Lentivirus	79787	500 µl x 2
ISRE Luciferase Reporter Lentivirus	79824	500 µl x 2
IL-2 Promoter Luciferase Reporter Lentivirus	79825	500 µl x 2
IL-8 Promoter Luciferase Reporter Lentivirus	79827	500 µl x 2
AP-1 Luciferase Reporter Lentivirus	79823	500 µl x 2
SBE Luciferase Reporter Lentivirus	79806	500 µl x 2
TEAD Luciferase Reporter Lentivirus	79833	500 µl x 2
ARE Luciferase Reporter Lentivirus	79869	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 (G418)	79692-G	500 µl x 2
Firefly Luciferase (Fluc) Lentivirus (Hygromycin)	79692-H	500 µl x 2
Firefly Luciferase (Fluc) Lentivirus (Puromycin)	79692-P	500 µl x 2