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. A variant called B.1.617.2 (also known as the Delta Variant) was identified in India in the spring of 2021. This variant has a number of mutations that increase morbidity and mortality and allow the virus to spread more easily and quickly than other variants.

The Spike (B.1.617.2 Variant) (SARS-CoV-2) Pseudotyped Lentiviruses were produced with SARS-CoV-2 B.1.617.2 Variant Spike (Genbank Accession #QHD43416.1 with B.1.617.2 mutations; see below for details) 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 Spike (B.1.617.2 Variant) (SARS-CoV-2) pseudotyped lentivirus can be used to measure the activity of neutralizing antibody against SARS-CoV-2 B.1.617.2 variant 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 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, the SARS-CoV-2 spike pseudotyped lentivirus does not transduce parental Calu3 and Vero E6 cells with great efficiency, as previously demonstrated by others [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].

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.

Spike Mutations in B.1.617.2 Variant:

T19R
G142D
156/157 DELETION
R158G
L452R
T478K
D614G
P681R
D950N

Applications

1. Study the mechanism of viral transduction
2. Screening for neutralizing antibodies for the delta variant of 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 and reagents 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
Spike S1 Neutralizing Antibody (Clone C-A11) (SARS-CoV-2)	BPS Bioscience, #101024
96-well tissue culture treated, white clear-bottom assay plate	Corning, #3610
ONE-Step™ luciferase assay system	BPS Bioscience, #60690

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, clear-bottom, 96-well microplate in 90 µl of Thaw Medium 1 (BPS Bioscience, #60187) (This step can be done during incubation of antibody and Spike pseudotyped lentivirus).

   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.

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

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

2. Day 3:

   Approximately 48-60 hours after transduction, prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 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

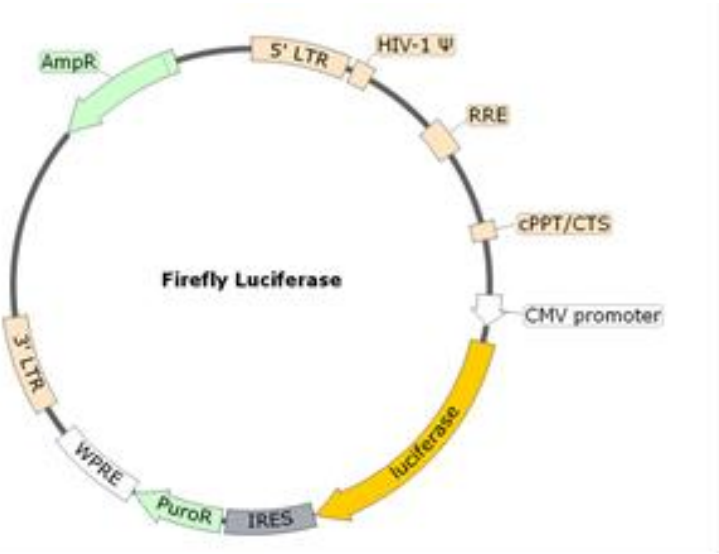

Figure 1. Schematic of the Luciferase Reporter in SARS-CoV-2 Spike (B.1.617.2 Variant) Pseudovirion

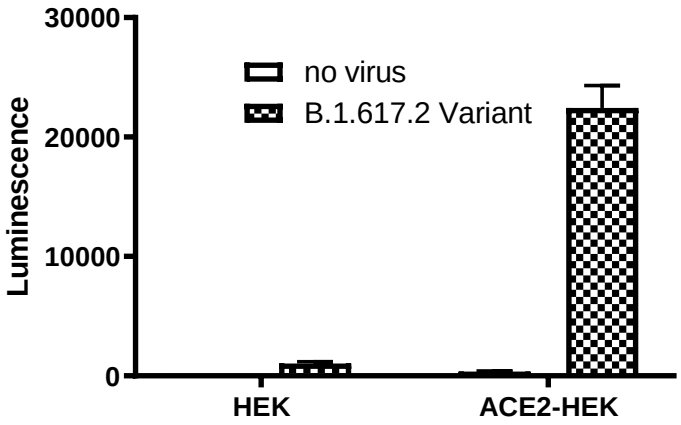

Figure 2. Transduction of ACE2-HEK293 cells
Approximately 8,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5 µl/well of Spike (B.1.617.2 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc reporter). After 48 hours of transduction, ONE-Step™ Luciferase reagent (BPS Bioscience, #60690) was added to cells to measure the luciferase activity. The Spike (B.1.617.2 Variant) (SARS-CoV-2) Pseudotyped Lentivirus transduced ACE2-HEK293 with much greater efficiency compared with HEK293 parental cells, indicating the transduction is dependent upon ACE2 expression.

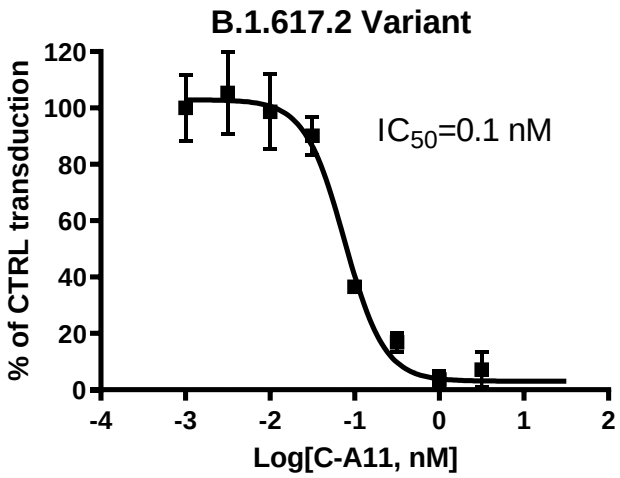

Figure 3: Neutralization assay by anti-SARS-CoV-2 Spike antibody
Approximately 8,000 ACE2-HEK293 cells/well were transduced with 10 µl/well of Spike (B.1.617.2 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc reporter)/anti-Spike antibody mix. After 48 hours of transduction, ONE-Step™ Luciferase reagent (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: C-A11) (BPS Bioscience, #101024) are shown.

Related Products

Products	*Catalog #*	*Size*
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x 2
ACE2 - HEK293 Recombinant Cell Line	79951	2 vials
Spike (B.1.617 Variant) Pseudotyped Lentivirus (Luc Reporter)	78204	500 µl x 2
Spike (B.1.617.1 Variant; Kappa) Pseudotyped Lentivirus (Luc Reporter)	78205	500 µl x 2
Spike (B.1.618 Variant) Pseudotyped Lentivirus (Luc Reporter)	78206	500 µl x 2
Spike (B.1.1.7 Variant; Alpha) (SARS-CoV-2) Pseudotyped lentivirus (Luc reporter)	78112	500 µl x 2
Spike (P.1 Variant; Gamma) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78144	500 µl x 2
Spike (B.1.429 Variant) Pseudotyped Lentivirus (Luc Reporter)	78172	500 µl x 2
Spike (B.1.351 variant; Beta) (SARS-CoV-2) Pseudotyped lentivirus (Luc reporter)	78142	500 µl x 2
Negative Control Lentivirus	79578	500 µl x 2