

## 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 B.1.429 variant (also known as **CAL.20C**) was identified in California in the spring of 2021 and contains a number of mutations. This variant may spread more easily and quickly than other variants.

The Spike (B.1.429 Variant) (SARS-CoV-2) Pseudotyped Lentiviruses were produced with SARS-CoV-2 B.1.429 Variant Spike (Genbank Accession #QHD43416.1 with B.1.429 variant 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.1.429 Variant) (SARS-CoV-2) pseudotyped lentivirus can be used to measure the activity of neutralizing antibody against SARS-CoV-2 B.1.429 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 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.

## Spike Mutations in B.1.429 Variant:

S13I  
W152C  
L452R  
D614G

## 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	<a href="#">BPS Bioscience #60187</a>
ACE2-HEK293 Recombinant Cell Line	<a href="#">BPS Bioscience #79951</a>
Spike S1 Neutralizing Antibody (Clone C-A11) (SARS-CoV-2)	<a href="#">BPS Bioscience #101024</a>
96-well tissue culture treated, white clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	<a href="#">BPS Bioscience #60690</a>

**Media Formulation**

*Thaw Medium 1 (BPS Bioscience #60187):*

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

## 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 90  $\mu$ l 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  $\mu$ l of the SARS-CoV-2 Spike pseudotyped lentivirus with 5  $\mu$ l of diluted anti-Spike antibody for 30 minutes. After incubation, add 10  $\mu$ l of virus/antibody mix into each well of the ACE2-HEK293 cells.

**To test anti-ACE2 antibody**, add 5  $\mu$ l 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  $\mu$ l of SARS-CoV-2 Spike pseudotyped lentivirus into each well.

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

Incubate the plates at 37°C with 5% CO<sub>2</sub>.

- ### 2. Day 3, approximately 48-60 hours after transduction, prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 $\mu$ l 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](https://bpsbioscience.com/license) for the label license and other key information about this product.

## Troubleshooting Guide

Visit [bpsbioscience.com/lentivirus-faq](https://bpsbioscience.com/lentivirus-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

Figures and Validation Data

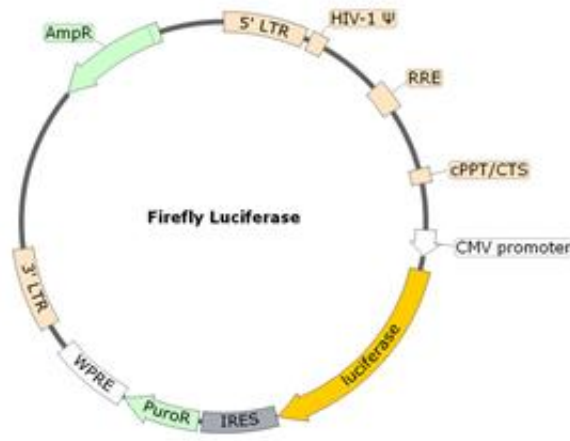


Figure 1. Schematic of the Luciferase Reporter in SARS-CoV-2 Spike Pseudovirion

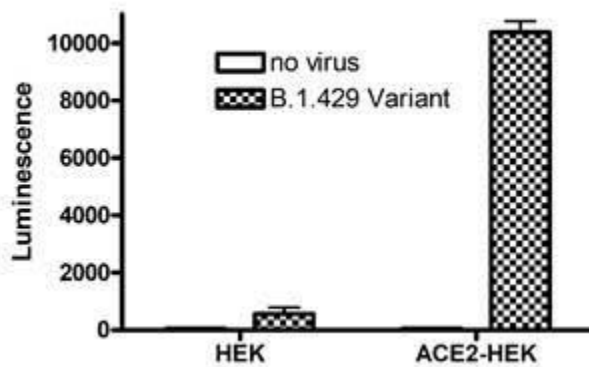
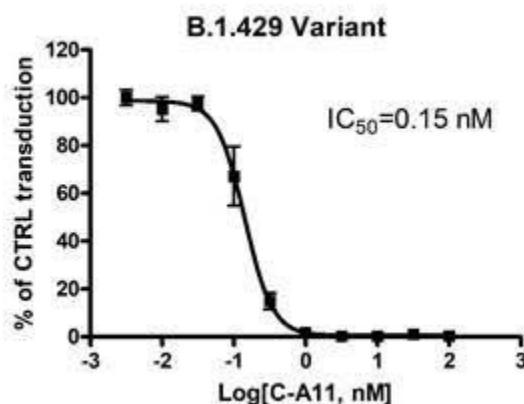


Figure 2. Transduction of ACE2-HEK293 cells

Approximately 8,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5  $\mu$ l/well of Spike (B.1.429 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.1.429 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  $\mu$ l/well of Spike (B.1.429 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 $\mu$ l x 2
Spike (B.1.351 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78160	500 $\mu$ l x 2
Spike (P.1 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78159	500 $\mu$ l x 2
Spike (B.1.1.7 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78158	500 $\mu$ l x 2
Spike Pseudotyped Lentivirus (eGFP Reporter)	79981	500 $\mu$ l x 2
Bald Lentiviral Pseudovirion (eGFP Reporter)	79987	500 $\mu$ l x 2
Spike (B.1.1.7 Variant) (SARS-CoV-2) Pseudotyped lentivirus (Luc reporter)	78112	500 $\mu$ l x 2
Spike (P.1 Variant) (SARS-CoV-2) Pseudotyped lentivirus (Luc reporter)	78144	500 $\mu$ l x 2
Spike (B.1.351 variant) (SARS-CoV-2) Pseudotyped lentivirus (Luc reporter)	78142	500 $\mu$ l x 2
ACE2-HEK293 Recombinant Cell Line	79951	500 $\mu$ l x 2
Spike Pseudotyped Lentivirus (Luciferase-eGFP Dual Reporter)	79982	500 $\mu$ l x 2