**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 human ACE2 may offer protection against the viral infection. Numerous SARS-CoV-2 variants have been identified so far. These variants contain a number of mutations that may increase morbidity and mortality and allow the virus to spread more easily and quickly than the original strain.

BPS Bioscience has launched a series of Spike Variants (SARS-CoV-2) Pseudotyped Lentivirus (Luc reporter). The Spike (SARS-CoV-2) Pseudotyped Lentiviruses were produced with SARS-CoV-2 Spike Variant (see below for mutation 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 Variants (SARS-CoV-2) Pseudotyped Lentivirus Pack (Luciferase Reporter) contains a collection of 12 Spike variants (SARS-CoV-2) Pseudotyped lentivirus (Luc reporter). It is a great tool to screen for variant-specific antibodies or to test the binding or efficacy of drug candidates against the different Spike variants. The Spike (SARS-CoV-2) pseudotyped lentiviruses can be used to measure the activity of neutralizing antibody against SARS-CoV-2 infection 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.

The pack provides one vial of each of the following:

| SARS-CoV-2 Variant | BPS CAT# | Sequence details |
|---|---|---|
| WT Spike Wuhan-Hu-1 | 79942-1 | Genbank Accession #QHD43416.1 |
| D614G | 78028-1 | D614G |
| B.1.1.7 (Alpha variant, UK) | 78112-1 | Deletions of H69, V70, and Y144; N501Y, A570D, D614G, P681H, T716I, S982A, D1118H |
| B.1.351 (Beta variant, SA) | 78142-1 | L18F, D80A, D215G, R246I, K417N, E484K, N501Y, D614G, A701V |
| P.1 (Gamma variant, Brazil) | 78144-1 | L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I |
| B.1.429 (Epsilon variant) | 78172-1 | S13I, W152C, L452R, D614G |
| B.1.617 | 78204-1 | L452R, E484Q, D614G, P681R |
| B.1.617.1 (Kappa variant) | 78205-1 | G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H |
| B.1.618 | 78206-1 | Y145del, H146del, E484K, D614G |
| B.1.617.2 (Delta variant) | 78215-1 | T19R, G142D, 156/157del, R158G, L452R, T478K, D614G, P681R, D950N |
| B.1.617.2.1 (Delta plus) | 78218-1 | T19R, G142D, 156/157del, R158G, K417N, L452R, T478K, D614G, P681R, D950N |
| B.1.621 (Mu variant) | 78618-1 | T95I, Y144S, Y145N, R346K, E484K, N501Y, D614G, P681H, D950N |

**Applications**

1. Study the mechanism of viral transduction
2. Screening for neutralizing antibodies that inhibit the interaction between ACE2 and the Spike protein of SARS-CoV-2 variants.
3. Identify variant-specific antibodies recognizing the different Spike mutations.

**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, #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 are seeded, but no virus or antibody is 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 48-72 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 is determined by measuring the luciferase activity.

**Figures and Validation Data**

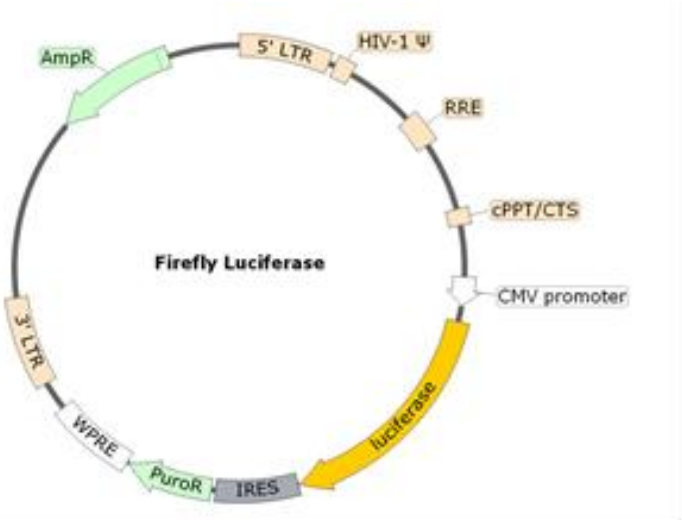


*Figure 1. Schematic of the Luciferase Reporter in Spike (SARS-CoV-2) Pseudotyped Lentiviruses*

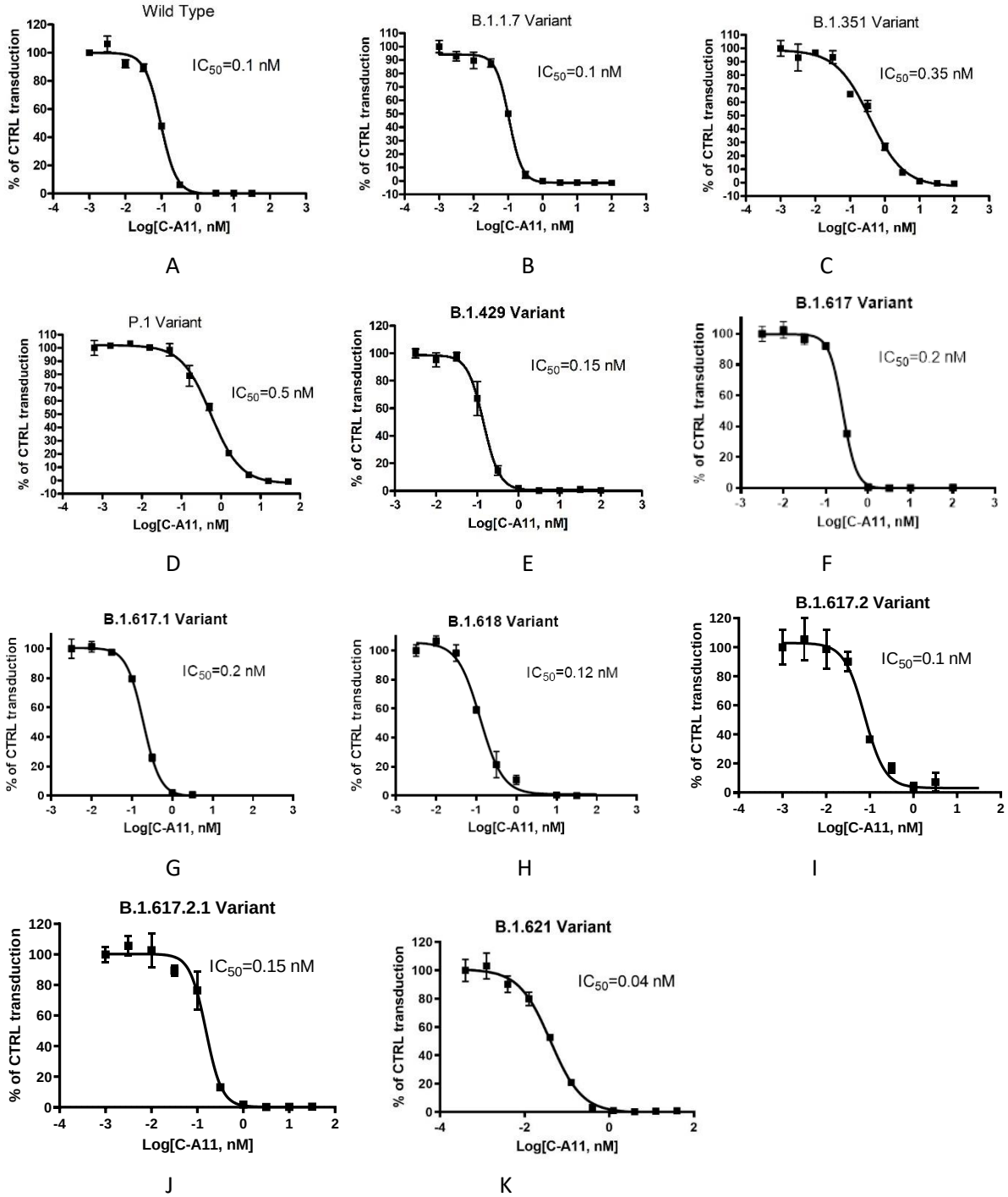


Figure 2: Representative titration plots of Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luc reporter) by anti-SARS-CoV-2 Spike antibody (clone# C-A11)

Approximately 8,000 ACE2-HEK293 cells/well were transduced with 10 µl/well of Spike (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 curve for Spike S1 Neutralizing Antibody (SARS-CoV-2) (Clone: C-A11) (BPS Bioscience, #101024) is shown.

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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* |
|---|---|---|
| Bald Lentiviral Pseudovirion (Luciferase Reporter) | 79943 | 500 µl x 2 |
| ACE2 - HEK293 Recombinant Cell Line | 79951 | 2 vials |
| Spike (B.1.617.2; Delta Variant) (SARS-CoV-2) Pseudotyped | 78215 | 500 µl x 2 |
| 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 |
| Spike (B.1.617.2.1; Delta Plus Variant) Pseudotyped Lentivirus (Luc Reporter) | 78218 | 500 µl x 2 |
| Spike (D614G) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter) | 78028 | 500 µl x 2 |
| Spike (B.1.617.2; Delta Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter) | 78216 | 500 µl x 2 |
| Spike (B.1.351 Variant) (SARS-CoV-2) Pseudotyped Lentivirus | 78160 | 500 µl x 2 |
| Spike (B.1.1.7 Variant) (SARS-CoV-2) Pseudotyped Lentivirus | 78158 | 500 µl x 2 |