

### 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 variant called B.1.351 was first identified in the fall of 2020 in the Republic of South Africa. This South African variant, also known as 501Y.V2, has many mutations which may lead to higher transmissibility and infectivity. The Spike (B.1.351 Variant) (SARS-CoV-2) Pseudotyped Lentivirus were produced with SARS-CoV-2 B.1.351 Variant Spike (Genbank Accession #QHD43416.1 with B.1.351 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.1.351 Variant) (SARS-CoV-2) Pseudotyped Lentivirus can be used to measure the activity of neutralizing antibody against SARS-CoV-2 B.1.351 variant in a Biosafety Level 2 facility.

### Spike Mutations in the B.1.351 Variant

L18F  
D80A  
D215G  
R246I  
K417N  
E484K  
N501Y  
D614G  
A701V

### Application

1. Study the mechanism of viral transduction of SARS-CoV-2 B.1.351 variant
2. Screening for neutralizing antibodies inhibiting the binding of SARS-CoV-2 Spike (B.1.351 variant) to ACE2.

### Formulation

The lentiviruses were produced from HEK293T cells. Supplied in medium containing 90% DMEM + 10% FBS.

### Titer

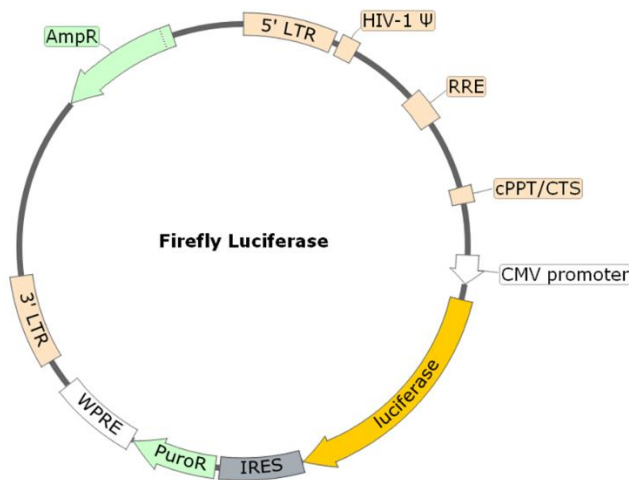
The titer will vary with each lot; the exact value is provided with each shipment.

**Storage**

Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus at  $-80^{\circ}\text{C}$ . 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.



**Figure 1. Schematic of the Luciferase Reporter in Spike (B.1.351 Variant) (SARS-CoV-2) Pseudotyped Lentivirus**

**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	<a href="#">BPS Bioscience, #60187</a>
Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luc reporter)	<a href="#">BPS Bioscience, #79942</a>
ACE2- HEK293 Recombinant Cell Line	<a href="#">BPS Bioscience, #79951</a>
Anti-SARS-CoV-2 Spike Neutralizing Antibody (Clone 414-2)	<a href="#">BPS Bioscience, #100792</a>
Anti-SARS-CoV-2 Spike Neutralizing Antibody (Clone 414-1)	<a href="#">BPS Bioscience, #100793</a>
96-well white clear-bottom assay plate	Corning, #3610
ONE-STEP Luciferase Assay System	<a href="#">BPS Bioscience, #60690</a>

### Assay Protocol

The following protocol is a general guideline for transducing ACE2-HEK293 cells using Spike (B.1.351 Variant) (SARS-CoV-2) 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<sub>2</sub> overnight.

To demonstrate transduction is dependent on ACE2, the same number of HEK293 parental cells can be 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 Spike (B.1.351 Variant) (SARS-CoV-2) 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 Spike (B.1.351 Variant) (SARS-CoV-2) Pseudotyped Lentivirus into each well.

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

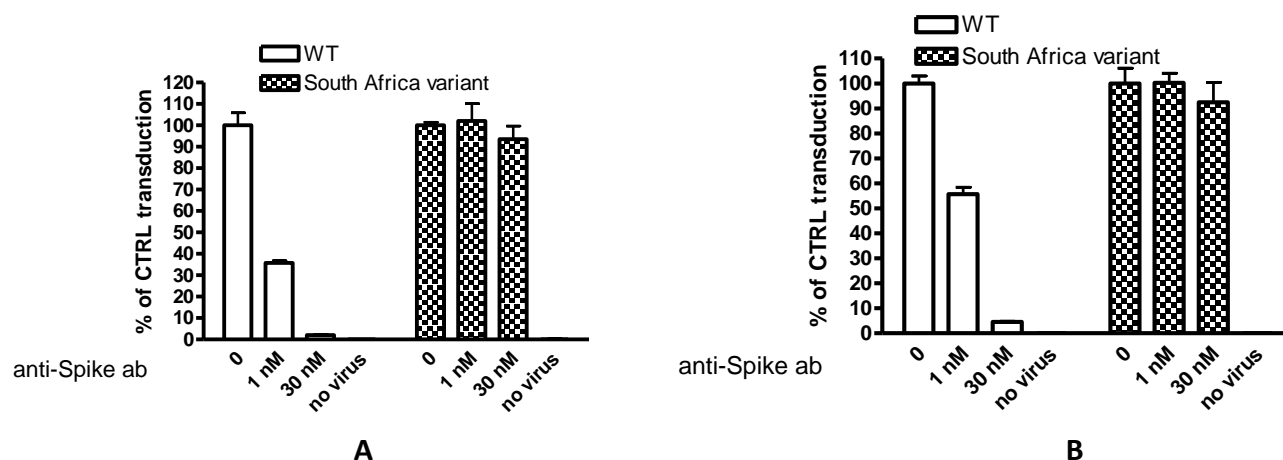
Incubate the plates at 37°C with 5% CO<sub>2</sub> overnight.

*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.



**Figure 2. Transduction of ACE2-HEK293 Cells using Spike (B.1.351 Variant) (SARS-CoV-2) Pseudotyped Lentivirus.**

Approximately 8,000 ACE2-HEK293 cells/well were transduced with 10  $\mu$ l/well of either wild type Spike (SARS-CoV-2) pseudotyped lentivirus (Luc reporter) (BPS Bioscience, #79942) or Spike (B.1.351 Variant) (SARS-CoV-2) pseudotyped lentivirus (Luc reporter) (BPS Bioscience, #78142) mixed with A) anti-Spike antibody (BPS Bioscience, #100793; clone#414-1) or B) anti-Spike antibody (BPS Bioscience, #100792; clone#414-2). After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience, #60690) was added to cells to measure the luciferase activity. Both antibodies inhibit the transduction of the wild-type Spike (SARS-CoV-2) pseudotyped lentivirus, but not the B.1.351 a variant.

#### License Disclosure

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#### 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).

#### Related Products

Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	79942	500 $\mu$ l x2
Spike (B.1.1.7 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78112	500 $\mu$ l x2
Spike (SARS-CoV-2, Brazil variant 1) Pseudotyped Lentivirus (Luc Reporter)	78143	500 $\mu$ l x2
Spike (SARS-CoV-2, Brazil variant 2) Pseudotyped Lentivirus (Luc Reporter)	78144	500 $\mu$ l x2
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 $\mu$ l x2
Spike Pseudotyped Lentivirus (eGFP Reporter)	79981	500 $\mu$ l x2
Bald Lentiviral Pseudovirion (eGFP Reporter)	79987	500 $\mu$ l x2
Spike Pseudotyped Lentivirus (Luciferase-eGFP Dual Reporter)	79982	500 $\mu$ l x2
Bald Lentiviral Pseudovirion (Luciferase-eGFP Dual Reporter)	79988	500 $\mu$ l x2
ACE2-HEK293 Recombinant Cell Line	79951	2 vials