

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.

In Brazil, a variant called P.1 was first identified in the summer of 2020. This variant has many mutations that may lead to higher transmissibility and infectivity. The Spike (P.1) (SARS-CoV-2) Pseudotyped Lentiviruses were produced with SARS-CoV-2 Variant Spike (Genbank Accession #QHD43416.1 with P.1 mutations, see below for details) as the envelope glycoproteins instead of the commonly used VSVG. These pseudovirions contain the enhanced green fluorescent protein (eGFP) gene driven by a CMV promoter (Figure 1), therefore, the spike-mediated cell entry can be determined via eGFP fluorescence. The Spike (P.1) (SARS-CoV-2) pseudotyped lentivirus can be used to measure the activity of neutralizing antibody against SARS-CoV-2 (P.1) variant using 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.

Spike Mutations in the P.1 Variant

L18F
T20N
P26S
D138Y
R190S
K417T
E484K
N501Y
D614G
H655Y
T1027I

Application

1. Study the mechanism of viral transduction of SARS-CoV-2 (P.1 variant)
2. Screening for neutralizing antibodies for SARS-CoV-2 Spike (P.1 variant) 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.

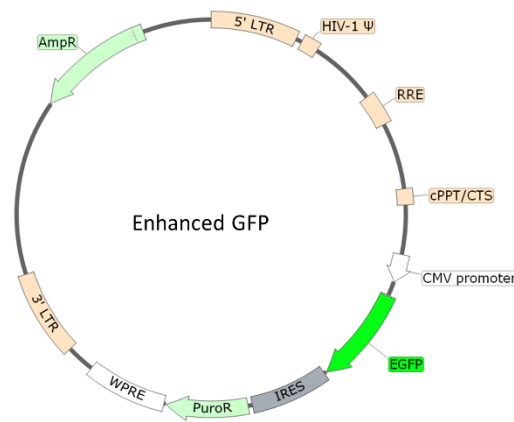


Figure 1. Schematic of the eGFP Reporter in Spike (P.1 Variant) (SARS-CoV-2) Pseudotyped Lentivirus

Materials Required but Not Supplied



These materials are not supplied with this lentivirus but are necessary to follow the designed protocol. BPS Bioscience media 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	BPS Bioscience, #60187
ACE2- HEK293 Recombinant Cell Line	BPS Bioscience, #79951
96-well white clear-bottom assay plate	Corning, #3610

Assay Protocol

The following protocol is a general guideline for transducing ACE2-HEK293 cells using Spike (P.1 Variant) (SARS-CoV-2) pseudotyped lentivirus (eGFP 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 50 μ l of Thaw Medium 1 (BPS Bioscience, #60187). Incubate cells at 37°C with 5% CO₂ 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: Add 20 μ l of Spike (P.1 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP reporter) into each well.

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

Incubate the plates at 37°C with 5% CO₂ 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 μ l of fresh Thaw Medium 1 to each well.

If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 3. The target cells can be incubated with the virus for 48-72 hours before changing medium.

4. Day 4-5, approximately 48-72 hours after transduction, the expression of eGFP in the target cells was examined by fluorescence microscopy.

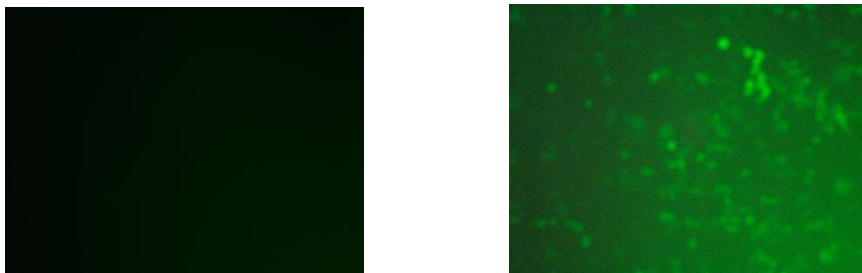


Figure 2. Transduction of ACE2-HEK293 cells using Spike (SARS-CoV-2, P.1 variant) pseudotyped lentivirus (eGFP reporter). Approximately 8,000 cells/well of ACE2-HEK293 cells (right) or HEK293 parental cells (left) were seeded and transduced on the same day with 20 μ l/well of Spike (SARS-CoV-2, P.1 variant) pseudotyped lentivirus (eGFP reporter). After 66 hours of transduction, the expression of eGFP in the target cells was observed under a fluorescence microscope.

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.

Related Products

Spike (B.1.351 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78160	500 µl x2
Spike (B.1.1.7 Variant) (SARS-CoV-2)Pseudotyped Lentivirus (eGFP Reporter)	78158	500 µl x2
Spike Pseudotyped Lentivirus (eGFP Reporter)	79981	500 µl x2
Bald Lentiviral Pseudoviron (eGFP Reporter)	79987	500 µl x2
Spike Pseudotyped Lentivirus (Luciferase-eGFP Dual Reporter)	79982	500 µl x2
Bald Lentiviral Pseudoviron (Luciferase-eGFP Dual Reporter)	79988	500 µl x2
Spike Pseudotyped Lentivirus (Luciferase Reporter)	79942	500 µl x2
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x2
Spike (B.1.1.7 Variant) (SARS-CoV-2) Pseudotyped lentivirus (Luc reporter)	78112	500 µl x2
Spike (P.1 Variant) (SARS-CoV-2) Pseudotyped lentivirus (Luc reporter)	78144	500 µl x2
Spike (B.1.351 variant) (SARS-CoV-2) Pseudotyped lentivirus (Luc reporter)	78142	500 µl x2
ACE2-HEK293 Recombinant Cell Line	79951	2 vials
Thaw Medium 1	60187	100 ml