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.1.617.2.1 (also known as the Delta Plus 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.1.617.2.1 Variant) (SARS-CoV-2) Pseudotyped Lentiviruses were produced with SARS-CoV-2 B.1.617.2.1 Variant Spike (Genbank Accession #QHD43416.1 with B.1.617.2.1 mutations; see below for details) as the envelope glycoproteins instead of the commonly used VSV-G. Compared to the Delta variant (B.1.617.2), variant Delta Plus has additional mutation K417N. These pseudovirions contain the enhanced green fluorescent protein (eGFP) reporter gene driven by a CMV promoter (Figure 1), therefore, the spike-mediated cell entry can be determined via eGFP fluorescence. The Spike (B.1.617.2.1 Variant) (SARS-CoV-2) pseudotyped lentivirus can be used to measure the activity of neutralizing antibody against SARS-CoV-2 B.1.617.2.1 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.

<u>Spike Mutations in B.1.617.2.1 Variant:</u>

T19R
G142D
156/157 DELETION
R158G
K417N
L452R
T478K
D614G
P681R
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 Delta Plus variant.

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.

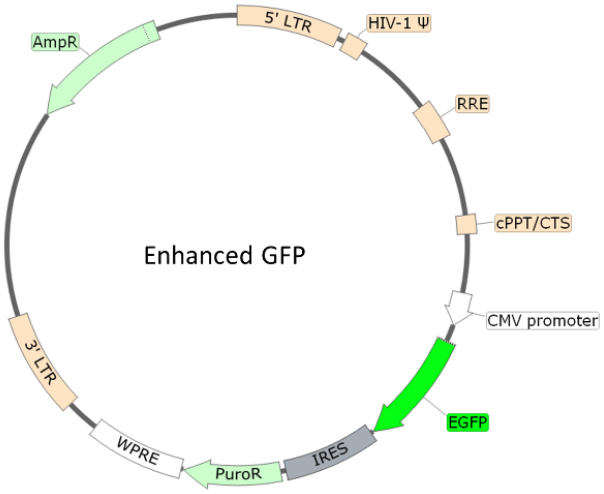

Figure 1. Schematic of the eGFP Reporter in Spike (B.1.617.2.1 Variant) (SARS-CoV-2) Pseudotyped Lentivirus

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 (B.1.617.2.1 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 µl of Thaw Medium 1 (BPS Bioscience, #60187). Add 10 µl of Spike (B.1.617.2.1 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP reporter) into each well.

   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, the expression of eGFP in the target cells is examined by fluorescence microscopy.

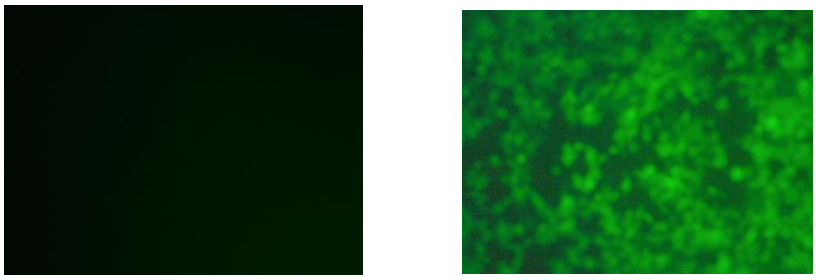

Figure 2. Transduction of ACE2-HEK293 cells using Spike (SARS-CoV-2, B.1.617.2.1 Variant) pseudotyped lentivirus (eGFP reporter). Approximately 5,000 cells/well of ACE2-HEK293 cells (right) or HEK293 parental cells (left) were seeded and transduced on the same day with 10 µl/well of Spike (SARS-CoV-2, B.1.617.2.1 variant) pseudotyped lentivirus (eGFP reporter). After 66 hours of transduction, the expression of eGFP in the target cells was observed under a fluorescence microscope (ex. 488 nm, em 525 nm).

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

Products	*Catalog #*	*Size*
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 (eGFP Reporter)	78160	500 µl x 2
Spike (B.1.1.7 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78158	500 µl x 2
Spike Pseudotyped Lentivirus (eGFP Reporter)	79981	500 µl x 2
Bald Lentiviral Pseudoviron (eGFP Reporter)	79987	500 µl x 2
Spike Pseudotyped Lentivirus (Luciferase-eGFP Dual Reporter)	79982	500 µl x 2
Bald Lentiviral Pseudoviron (Luciferase-eGFP Dual Reporter)	79988	500 µl x 2
Spike Pseudotyped Lentivirus (Luciferase Reporter)	79942	500 µl x 2
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x 2
Spike (B.1.617.2; Delta Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78215	500 µl x 2
Spike (B.1.617 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78204	500 µl x 2
Spike (B.1.1.7 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78112	500 µl x 2
Spike (P.1 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78144	500 µl x 2
Spike (B.1.351 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78142	500 µl x 2
ACE2-HEK293 Recombinant Cell Line	79951	2 vials
Thaw Medium 1	60187	100 ml