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 Omicron Variant was identified in South Africa in November of 2021. This variant has a large number of mutations that allow the virus to spread more easily and quickly than other variants. As of February 2022, Omicron variants have been divided into four distinct sub-lineages: BA.1 (B.1.1.529), BA.1.1, BA.2, and BA.3. Compared with BA.1 (B.1.1.529), BA.1.1 has an additional R346K substitution in the spike protein.

The Spike (BA.1.1, Omicron Variant R346K) (SARS-CoV-2) Pseudotyped Lentiviruses were produced with SARS-CoV-2 BA.1.1 Variant Spike (Genbank Accession #QHD43416.1 containing all the BA.1.1 mutations; see below for details) as the envelope glycoproteins instead of the commonly used VSV-G. These pseudovirions contain the eGFP gene driven by a CMV promoter (Figure 1), therefore, the spike-mediated cell entry can be determined via eGFP fluorescence. The Spike (BA.1.1, Omicron Variant R346K) (SARS-CoV-2) pseudotyped lentivirus can be used to measure the activity of neutralizing antibody against SARS-CoV-2 BA.1.1 variant in a Biosafety Level 2 facility.

As shown in Figures 2, the Spike Omicron pseudovirus has been validated for use with target cells ACE2-HEK293 (which overexpress ACE2; BPS Bioscience #79951).

Spike Mutations in BA.1.1 Omicron Variant R346K:
A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, R346K, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F

Applications

Screening for neutralizing antibodies for the SARS-CoV-2 BA.1.1 variant in ACE2-HEK293 cells

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.

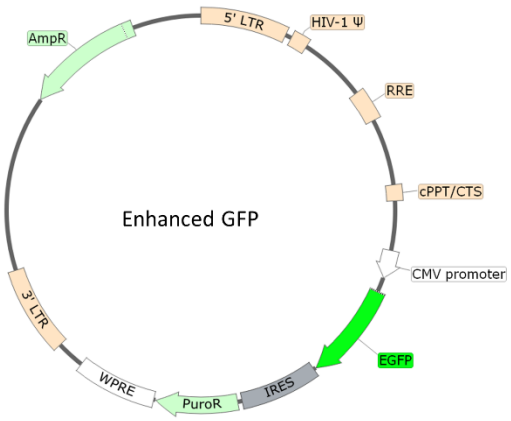

Figure 1. Schematic of the eGFP Reporter in Spike (BA.1.1, Omicron Variant R346K) (SARS-CoV-2) Pseudotyped Lentivirus

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 (BA.1.1, Omicron Variant R346K) (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 5-10 µl of Spike (BA.1.1, Omicron Variant R346K) (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 was examined by fluorescence microscopy.

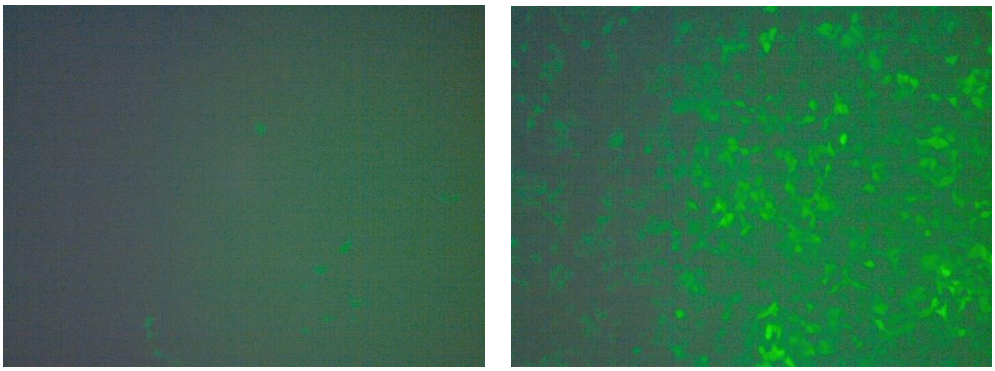

Figure 2. Transduction of ACE2-HEK293 cells using Spike (BA.1.1, Omicron Variant R346K) Pseudotyped Lentivirus (eGFP Reporter). Approximately 5,000 cells/well of ACE2-HEK293 cells (right) or HEK293 parental cells (left) were seeded and transduced on the same day with 5 µl/well of Spike (BA.1.1, Omicron Variant R346K) pseudotyped lentivirus (eGFP reporter). After 66 hours of transduction, the expression of eGFP in the target cells was observed under a fluorescence microscope.

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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 Variant) Pseudotyped Lentivirus (Luc Reporter)	78204	500 µl x 2
Spike (B.1.617.1, Kappa Variant) 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, Alpha Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78112	500 µl x 2
Spike (B.1.429, Epsilon Variant) Pseudotyped Lentivirus (Luc Reporter)	78172	500 µl x 2
Spike (B.1.351, Beta Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78142	500 µl x 2
Spike (B.1.617.2; Delta Variant) Pseudotyped Lentivirus (Luc Reporter)	78215	500 µl x 2
Spike (B.1.1.529 BA.1, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78348	500 µl x 2
Spike (BA.2, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78625	500 µl x 2
Spike (B.1.1.529 BA.1.1, Omicron Variant R346K) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78623	500 µl x 2