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Description

The Spike (XBB.1.16 Omicron Variant) (SARS-CoV-2) Pseudotyped Lentiviruses are replication incompetent, HIV-based lentiviral particles. They were produced with SARS-CoV-2 Spike (Genbank Accession #QHD43416.1 containing all the Omicron XBB.1.16 mutations; see below for details) as the envelope glycoprotein instead of the commonly used VSV-G. These pseudovirions contain eGFP driven by a CMV promoter (Figure 1), allowing the spike-mediated cell entry to be measured by the eGFP fluorescence signal. The Spike (XBB.1.16, Omicron Variant) (SARS-CoV-2) pseudotyped lentivirus can be used to measure the activity of neutralizing antibody against SARS-CoV-2 XBB.1.16.

The Spike Omicron XBB.1.16 pseudoviruses have been validated for use with ACE2-HEK293 target cells (which overexpress ACE2; BPS Bioscience #79951).

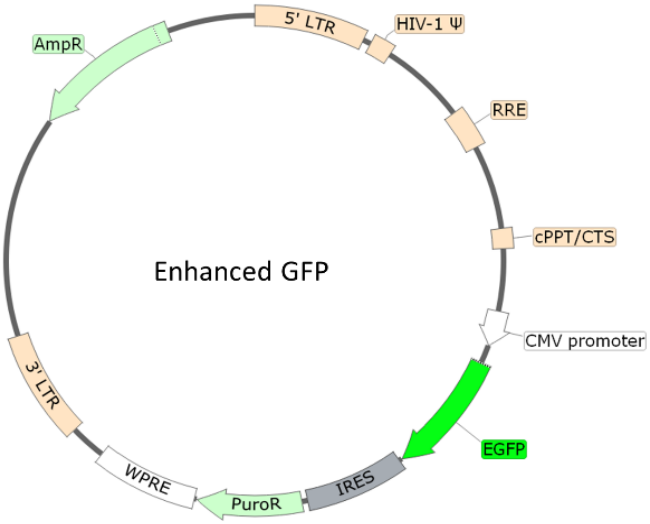

Figure 1. Schematic of the lenti-vector used to generate the eGFP Reporter in the Spike (XBB.1.16, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus.

Background

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. 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 easier and quicker than other variants. As of May 2022, Omicron variants were divided into seven distinct sub-lineages: BA.1, BA.1.1, BA.2, BA.3, BA.2.12.1, BA.4, and BA.5. As of April 2023, additional new sub-lineages (BQ.1, BQ.1.1, BF.7, XBB.1, XBB.1.5, XBB.1.16) have been identified.

Spike Mutations in XBB.1.16 Omicron Variant:
T19I, LPP24-26del, A27S, V83A, G142D, Y144del, H146Q, E180V, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478R, E484A, F486P, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K

Application(s)

Screen or titrate neutralizing antibodies against the SARS-CoV-2 XBB.1.16 variant in ACE2-expressing cells.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Size and Titer

The titer will vary with each lot; the exact value is provided with each shipment. Based on experiments performed by scientists at BPS Bioscience, 78785-1 (100 µl) provides sufficient signal-to-noise ratio to perform 100 reactions, and 78785-2 (500 µl x 2) for 1000 reactions. The amount of virus added to the cells can be titrated further down according to the user's need.

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 Used in the Validation Assay 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 (XBB.1.16, Omicron 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 can be measured approximately 48-72 hours after transduction.

Day 1:

1. Plate ACE2-HEK293 cells at a density of 5,000-10,000 cells per well in 50 µl of Thaw Medium 1 into white clear-bottom 96-well microplate.

2. Thaw the pseudovirus at Room Temperature (RT).

3. Add 1-5 µl of Spike (XBB.1.16, Omicron 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.

4. Incubate the plates at 37°C with 5% CO_2.

Day 3:

1. Approximately 48-72 hours after transduction, examine the expression of eGFP in the target cells by fluorescence microscopy, or other appropriate technique.

Validation Data

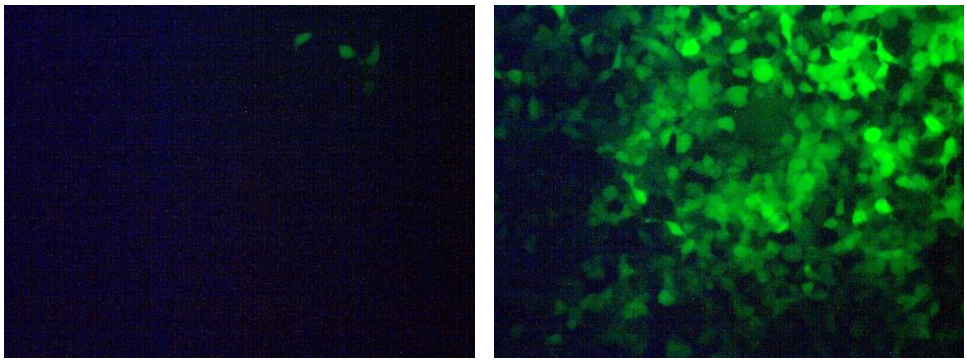

Figure 2. Fluorescence microscopy of ACE2-HEK293 cells transduced with Spike (XBB.1.16, Omicron Variant) Pseudotyped Lentivirus (eGFP Reporter).
Approximately 5,000 cells/well of ACE2-HEK293 cells (right) or HEK293 parental cells (left) were seeded and transduced on the same day with 1 µl/well of Spike (XBB.1.16, Omicron variant) pseudotyped lentivirus (eGFP reporter). After 66 hours of transduction, the expression of eGFP in the target cells was observed under a fluorescence microscope. The Spike Pseudotyped Lentivirus transduced ACE2-HEK293 have a greater transduction efficiency, compared with HEK293 parental cells, indicating the transduction is dependent upon ACE2 presence.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

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 (BA.2, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78626	500 µl x 2
Spike (B.1.1.529 BA.1, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78349	500 µl x 2
Spike (BA.1.1, Omicron Variant R346K) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78624	500 µl x 2