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 XBB.1.16 mutations; see below for details) as the envelope glycoprotein instead of the commonly used VSV-G. These pseudovirions contain firefly luciferase driven by a CMV promoter (Figure 1), allowing the spike-mediated cell entry to be measured by luciferase activity. The Spike (XBB.1.16, Omicron Variant) (SARS-CoV-2) pseudoviruses can be used to measure the activity of a neutralizing antibody against the SARS-CoV-2 Omicron XBB.1.16 variant.

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

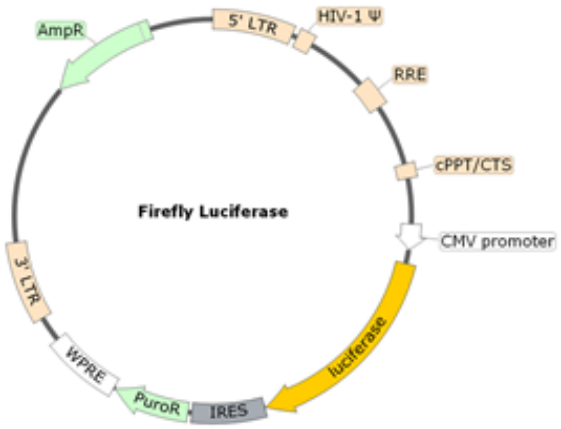

Figure 1. Schematic of the lenti-vector used to generate the Luciferase Reporter in the Spike (XBB.1.16, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus.

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 SARS-CoV-2 Spike Omicron 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, 78784-1 (100 µl) provides sufficient signal-to-noise ratio to perform 100 reactions, and 78784-2 (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 lentiviruses 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 and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience 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 protocol described in the "Validation Data" section. Media, reagents, and luciferase assay buffers used at BPS Bioscience 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
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture treated, white clear-bottom assay plate	Corning #3610

Assay Protocol

- The following protocol is a general guideline for transducing ACE2-HEK293 cells using SARS-CoV-2 Spike 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 can be measured approximately 48-72 hours after transduction.
- The luminescence reading can be influenced by multiple factors including cell type, detection reagent or luminometer. To maximize the use of the virus, a pre-test can be carried out to determine the optimal virus dosage per well. The pseudovirus can be diluted with Thaw Medium 1. In general, we recommend a 5-fold dilution.

Day 1:

1. Plate ACE2-HEK293 cells at a density of 5,000-10,000 cells per well in 90 µl of Thaw Medium 1 into white, clear-bottom, 96-well microplate. (This step can be done during incubation of the antibody with Spike pseudotyped lentivirus).

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

3. Dilute the pseudoviruses with Thaw Medium 1 according to your pretest results.

4. Prepare serial dilutions of anti-Spike or anti-ACE2 antibody in Thaw Medium 1.

    a. To test an anti-Spike antibody, preincubate 5 µl of diluted SARS-CoV-2 Spike pseudotyped lentiviruses with 5 µl of diluted anti-Spike antibody for 30 minutes.

    After incubation, add 10 µl of the virus/antibody mix into each well containing ACE2-HEK293 cells.

    b. To test an anti-ACE2 antibody, add 5 µl of diluted anti-ACE2 antibody to the ACE2-HEK293 cells and incubate for 30 minutes. Add medium only to the “No-Antibody” positive control.

    At the end of the incubation, add 5 µl of diluted SARS-CoV-2 Spike pseudotyped lentivirus into each well.

5. For control wells, seed the same number of ACE2-HEK293 cells but do not add virus or antibody.

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

Day 3:

1) Approximately 48-66 hours after transduction, add 100 µl of ONE-Step™ Luciferase Assay reagent per well.

2) 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.

Validation Data

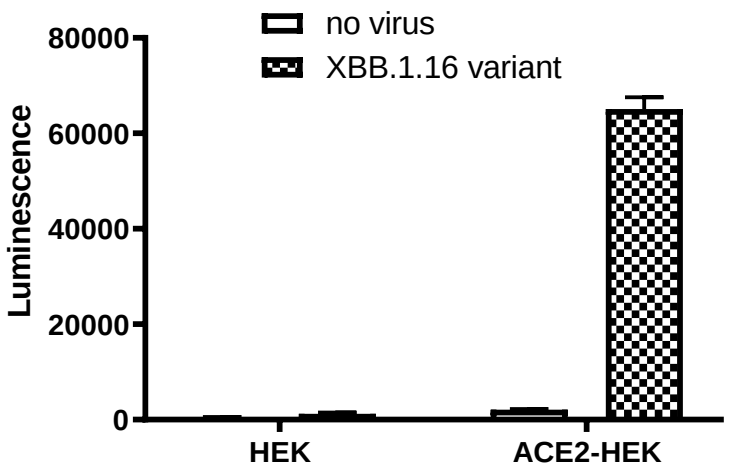

Figure 2. Luciferase activity of ACE2-HEK293 cells transduced with Spike (XBB.1.16, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luciferase reporter).
Approximately 8,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5 µl of 5-fold dilution of Spike (XBB.1.16, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luciferase reporter). After 66 hours of transduction, ONE-Step™ Luciferase Assay System was added to cells to measure the luciferase activity. 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.4/5, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78646	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 (B.1.1.529 BA.1; Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78349	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
Spike (BA.2, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78625	500 µl x 2