Description

The Spike (JN.1, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luciferase Reporter) are replication incompetent, HIV-based lentiviral particles. They were produced with SARS-CoV-2 Spike (Genbank Accession #QHD43416.1 containing all the JN.1 mutations; see below for details) as the envelope glycoprotein, instead of the commonly used VSV-G. These pseudovirions also contain a firefly luciferase reporter driven by a CMV promoter (Figure 1), allowing to measure spike-mediated cell entry using luciferase activity.

These pseudoviruses have been validated in a cellular assay with ACE2-HEK293 Recombinant Cell Line (#79951), a cell line that overexpresses ACE2 at high levels, as target cell line.

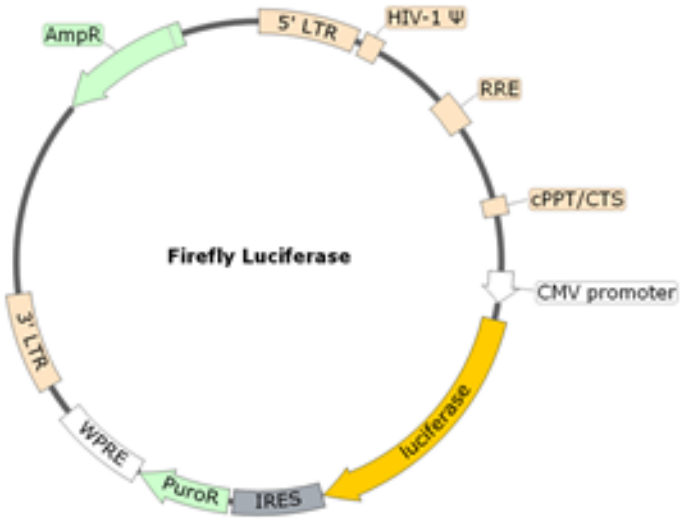

Figure 1. Schematic of the lenti-vector used to introduce the luciferase reporter in Spike (JN.1, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luciferase Reporter).

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 ACE2 may offer protection against the viral infection.

<u>Spike Mutations in JN.1 Omicron Variant:</u>

T19I, LPP24-26del, A27S, S50L, HV69-70del, V127F, G142D, Y144del, F157S, R158G, N211del, L212I, V213G, L216F, H245N, A264D, I332V, G339H, K356T, S371F, S373P, S375F, T376A, R403K, D405N, R408S, K417N, N440K, V445H, G446S, N450D, L452W, L455S, N460K, S477N, T478K, N481K, V483del, E484K, F486P, Q498R, N501Y, Y505H, E554K, A570V, D614G, P621S, H655Y, N679K, P681R, N764K, D796Y, S739F, Q954H, N969K, P1143L

Application(s)

Screen or titrate neutralizing antibodies against SARS-CoV-2 Spike Omicron JN.1 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, 78981-1 (100 µl size) provides sufficient pseudovirions to create a signal-to-noise ratio that allows to perform 100 reactions, and 78981-2 (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 plate	Corning #3610

Assay Protocol

- The following protocol is a general guideline for transducing ACE2-HEK293 cells using these pseudovirions. 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.
- Luminescence readings can be influenced by multiple factors including cell type, detection reagent or luminometer. We recommend using ONE-Step™ Luciferase Assay System for best results.
- To maximize the use of the virus, it is recommended that a pretest is performed 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.
- Testing should include "No Antibody", "No Virus" and "Test Antibody" conditions.

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 tissue culture plate.

   Note: This step can be done during the incubation of the test antibody with the Spike (JN.1, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luciferase Reporter).

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

3. Dilute the pseudoviruses with Thaw Medium 1 based on your pretest results.

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

   4.1 To test an anti-Spike antibody:

   i) Preincubate 5 µl of diluted pseudovirions with 5 µl of diluted anti-Spike antibody for 30 minutes.

   ii) Add 10 µl of the pseudovirions/antibody mix into each well containing ACE2-HEK293 cells ("Test Antibody" wells).

   iii) Add 10 µl of media to the "No Antibody" and "No Virus" wells.

   4.2 To test an anti-ACE2 antibody:

   i) Add 5 µl of diluted anti-ACE2 antibody to the ACE2-HEK293 cells ("Test Antibody" and "No Virus" wells).

   ii) Add 5 µl of medium only to the "No Antibody" positive control.

   iii) Incubate for 30 minutes.

   iv) Add 5 µl of diluted pseudotyped lentivirus into all wells, except the "No Virus" control wells.

   v) Add 5 µl of medium only to the "No Virus" wells.

5. Incubate the plates at 37°C with 5% CO_2 for 48-66 hours.

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.

3. Measure luminescence using a luminometer. The transduction efficacy is determined by measuring the luciferase activity.

Validation Data

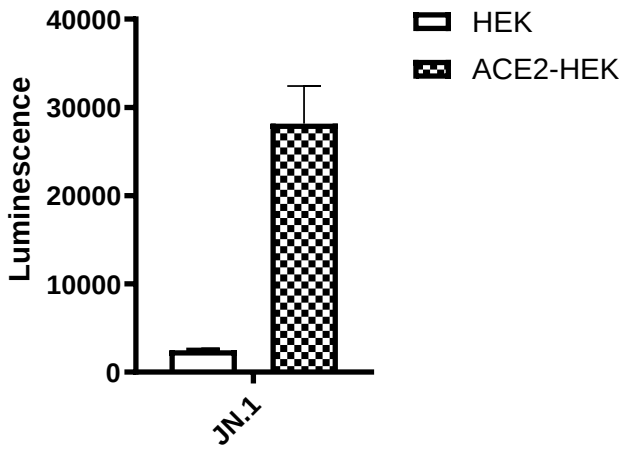

Figure 2. Luciferase activity of ACE2-HEK293 cells transduced with Spike (JN.1, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luciferase Reporter).
Approximately 8,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5 µl of a 5-fold dilution of Spike (JN.1, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luciferase Reporter). 66 hours post-transduction, ONE-Step™ Luciferase Assay System was added to cells to measure the luciferase activity. ACE2-HEK293 cells result in a greater transduction efficiency, compared with HEK293 parental cells, pointing to an ACE2 dependent transduction.

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