Description

The Spike (BA.1.1, Omicron Variant) (SARS-CoV-2) Pseudotyped VSV Delta G (Luciferase Reporter) was produced with SARS-CoV-2 Spike (Genbank Accession #QHD43416.1 containing all the Omicron BA.1.1 mutations; see below for details) as the envelope glycoprotein instead of VSV-G. The pseudovirions contain the firefly luciferase gene; therefore, the spike-mediated cell entry can be measured via luciferase activity. The Spike (BA.1.1 Variant) (SARS-CoV-2) Pseudotyped VSV Delta G (Luciferase Reporter) can be used to measure the activity of a neutralizing antibody against SARS-CoV-2 BA.1.1 variant in a Biosafety Level 2 facility.

As shown in Figures 1 and 2, the Spike (BA.1.1 Variant) (SARS-CoV-2) Pseudotyped VSV Delta G (Luciferase Reporter) has been validated for use with target cells Vero-E6 and ACE2-HEK293 (BPS Bioscience #79951). Spike VSV Delta G is preferred over lentiviral-based spike pseudoviruses for use in cells such as Vero-E6 parental cells.

Spike Mutations in BA.1.1 Omicron Variant:
A67V, Δ69-70, 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, T95I, Q954H, N969K, L981F

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 viral infection. The Omicron Variant was identified in South Africa in November of 2021. This variant has several mutations that allow the virus to spread more easily and quickly than other variants. As of May 2022, Omicron variants have been divided into seven distinct sub-lineages: BA.1, BA.1.1, BA.2, BA.3, BA.2.12.1, BA.4, and BA.5. Compared with BA.1 (B.1.1.529), BA.1.1 has an additional R346K substitution in the spike protein.

Vesicular stomatitis virus (VSV) is an enveloped, negative-stranded RNA virus that infects a wide range of animals and less frequently humans, causing mild flu-like symptoms. Its simple structure and its ability to grow in most mammalian cell types has made VSV a valuable tool to study virus entry, replication, and assembly. The glycoprotein of VSV (VSV-G), which binds to the LDL-receptor (low-density lipoprotein receptor), is responsible for the attachment and entry of VSV into a susceptible host cell. Recombinant VSV in which the glycoprotein was deleted (VSV Delta G) can accept viral envelop proteins from a variety of other viruses, allowing to generate pseudotypes that represent robust models to screen for neutralizing antibodies and other inhibitors of virus entry. The pseudoviruses can be engineered to transduce a reporter gene such as Firefly Luciferase or a fluorescent protein, so that viral entry can be monitored using luminescence or fluorescence.

The Spike (BA.1.1 Variant) (SARS-CoV-2) Pseudotyped VSV Delta G (Luciferase Reporter) virus contains BA.1.1 variant SARS-CoV-2 Spike instead of VSV-G, which allows the pseudovirus to bind to ACE2-expressing cells.

Application

- Screen or titrate neutralizing antibodies against SARS-CoV-2 Spike protein
- Model virus entry in Vero-E6 and ACE2-HEK293 cells

Formulation

The pseudoviruses were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

Titer

The titer will vary with each lot; the exact value is provided with each shipment. The minimal titer is >10^6 TU/ml.

Storage

The VSV Delta G pseudovirus is 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

The infectivity of VSV Delta G is restricted to a single round of replication. The pseudovirus can be handled using Biosafety Level 2 containment practices. BPS Bioscience recommends following all local, federal, state, and institutional regulations and using all appropriate safety precautions.

Materials Required but Not Supplied

These materials are not supplied with this pseudovirus 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 pseudovirus and are highly recommended for best results.

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Bald VSV Delta G (Luciferase reporter)	BPS Bioscience #78636
Vero-E6	ATCC #CRL-1586
ACE2-HEK293 Recombinant Cell Line	BPS Bioscience #79951
Spike Neutralizing Antibody (Clone G10xA1) (SARS-CoV-2)	BPS Bioscience #101326
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 Vero-E6 cells using Spike (BA.1.1 Variant) pseudotyped VSV Delta G (Luciferase reporter). The MOI should be optimized according to the cell types. A pre-test can be carried out to determine the preferred virus dosage per well. The pseudovirus can be diluted with DMEM medium + 10% FBS or Thaw Medium 1 (BPS Bioscience #60187).

For Vero-E6 cells, we recommend a 10-fold dilution, and the amount of virus added to the cells can be titrated further according to the user's need.

Day 1:

Vero-E6 cells were plated at a density of 20,000 cells per well into a white, clear-bottom 96-well microplate, in 90 µl of Thaw Medium 1. Cells were incubated at 37°C with 5% CO_2 overnight.

Day 2:

1. The pseudovirus was thawed at room temperature and diluted with Thaw Medium 1 according to the pretest results.

2. Serial dilutions of anti-Spike antibody were prepared in Thaw Medium 1.

To test an anti-Spike antibody, 5 µl of the diluted pseudovirus was preincubated with 5 µl of the diluted anti-Spike antibody for 30 minutes. After incubation, 10 µl of virus/antibody mix was added into each well of the Vero-E6 cells.

3. For control wells, the same number of cells were seeded, but no virus or antibody was added. The plates were incubated at 37°C with 5% CO_2.

Day 3:
Approximately 16-24 hours after transduction, the transduction efficacy was determined by measuring the luciferase activity. The ONE-Step™ Luciferase reagent was prepared per the recommended protocol. 100 µl of ONE-Step™ Luciferase Assay reagent was added per well and incubated at room temperature for ~15 to 30 minutes before measuring luminescence using a luminometer. “Blank” value was subtracted from all readings.

Figures and Validation Data

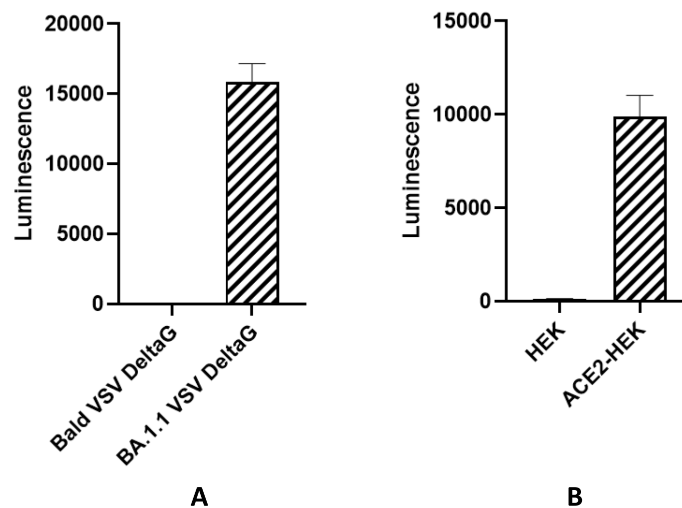

Figure 1. Transduction of Vero-E6 and ACE2-HEK293 cells.
Approximately 20,000 cells/well of Vero-E6 (A) or ACE2-HEK293 cells (B) were seeded in a 96-well white clear-bottom assay plate. The next day, cells were infected with 5 µl (prediluted 5-fold) of Spike (BA.1.1 variant) (SARS-CoV-2) pseudotyped VSV Delta G (Luciferase reporter). After 18 hours of transduction, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to cells to measure luciferase activity. Bald VSV Delta G (Luciferase reporter) (BPS Bioscience #78636) or HEK293 cells were used as negative controls.

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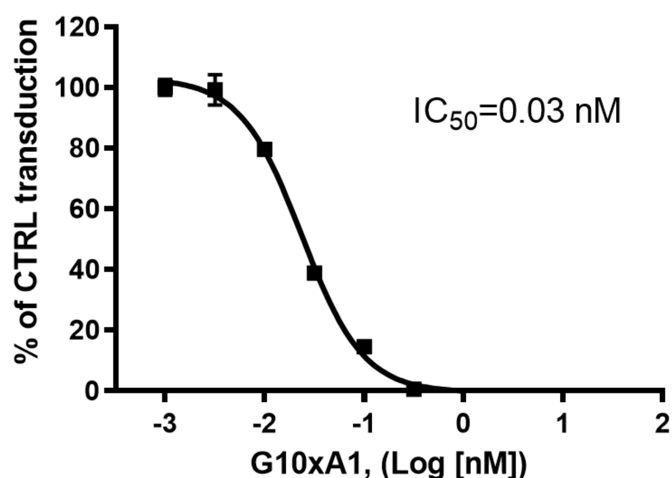

Figure 2. Neutralization assay using anti-SARS-CoV-2 Spike antibody.
Approximately 20,000 cells/well of Vero-E6 cells were seeded in a 96-well white clear-bottom assay plate. The next day, cells were infected with Spike (BA.1.1 variant) pseudotyped VSV Delta G (Luciferase reporter)/anti-Spike (Clone G10xA1) mix. After 18 hours of transduction, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure luciferase activity. The transduction efficiency of the wells with virus only (no antibody treatment) was set as 100%, while the transduction efficiency of the wells without virus was set as 0%. The titration curve for Spike Neutralizing Antibody (SARS-CoV-2) Clone G10xA1 (BPS Bioscience #101326) is shown.

References

Whitt MA. 2010. "Generation of VSV pseudotypes using recombinant ΔG-VSV for studies on virus entry, identification of entry inhibitors, and immune responses to vaccines." *J. Virol. Methods* **169(2)**: 365-74.

Troubleshooting Guide

For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Bald VSV Delta G (Luciferase Reporter)	78636	500 µl x 2
Spike (SARS-CoV-2) Pseudotyped VSV Delta G (Luciferase Reporter)	78637	500 µl x 2
VSV-G Pseudotyped VSV Delta G (Luciferase Reporter)	78634	500 µl x 2
Spike (SARS-CoV-2, BA.2) Pseudotyped VSV Delta G (Luciferase Reporter)	78635	500 µl x 2
Spike (SARS-CoV-2, BA.2.12.1) Pseudotyped VSV Delta G (Luciferase Reporter)	78643	500 µl x 2

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