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Description

The VSV-G Pseudotyped VSV Delta G (Luciferase Reporter) was produced by re-expression of VSV-G as the envelope glycoprotein using the VSV Delta G system in which VSV-G is deleted. The pseudovirions contain the firefly luciferase gene; therefore, the VSV-G mediated cell entry can be measured via luciferase activity. The VSV-G Pseudotyped VSV Delta G (Luciferase Reporter) can be used as a positive control of transduction for other VSV pseudotypes containing the envelope glycoproteins of heterologous viruses in a Biosafety Level 2 facility.

Background

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.

Application

Serve as a positive control of VSV transduction for VSV pseudotypes containing the heterologous envelope glycoproteins.

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 $>10^8$ 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
Spike (SARS-CoV-2) (BA.2, Omicron Variant) pseudotyped VSV Delta G (Luciferase Reporter)	BPS Bioscience #78635
Vero-E6	ATCC #CRL-1586
ACE2-HEK293 Recombinant Cell Line	BPS Bioscience #79951
Spike Neutralizing Antibody (Clone G10xA1) (SARS-CoV-2)	BPS Bioscience #101326
Anti-VSV-G Antibody (Clone 8G5F11)	MilliporeSigma #MABF2337
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 VSV-G pseudotyped VSV Delta G (Luciferase reporter). The MOI should be optimized according to the cell type. A pre-test can be carried out to determine the virus dosage per well. The pseudovirus can be diluted with DMEM medium + 10% FBS.

The titer of VSV-G pseudotyped VSV Delta G is much higher than spike (SARS-CoV-2) pseudotyped VSV Delta G. A dose where VSV-induced cytopathic effects (CPE) is not observed should be chosen for neutralization studies.

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 (BPS Bioscience #60187). Cells were incubated at 37°C with 5% CO_2 overnight.

Day 2:

1. The pseudovirus was thawed at room temperature and diluted with DMEM medium + 10% FBS according to the pretest results.

2. Serial dilutions of neutralization antibody were prepared in Thaw Medium 1.

   To test an anti-VSV-G antibody, 5 µl of the diluted pseudovirus was preincubated with 5 µl of the diluted anti-VSV-G 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

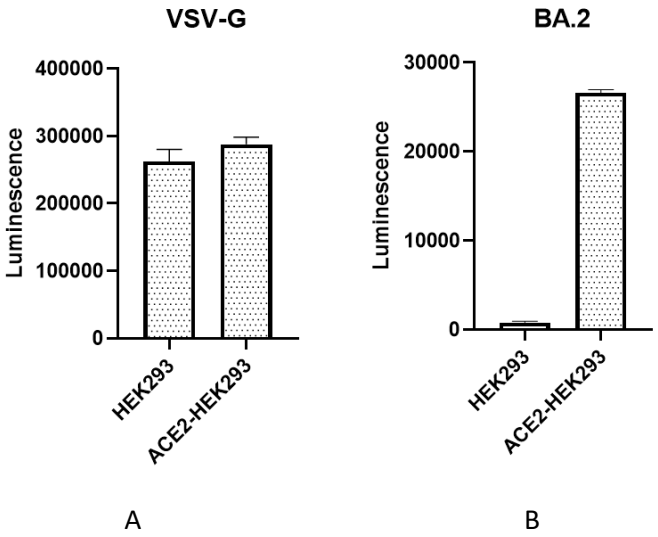

Figure 1. Transduction of HEK293 and ACE2-HEK293 cells.
Approximately 20,000 cells/well of HEK293 cells and ACE2-HEK293 cells were seeded in a 96-well white clear-bottom assay plate. The next day, cells were infected with VSV-G pseudotyped VSV Delta G (Luciferase reporter) (A) or Spike (SARS-CoV-2, BA.2) pseudotyped VSV Delta G (Luciferase reporter) (B). After 18 hours of transduction, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to cells to measure luciferase activity. VSV-G pseudotyped VSV Delta G transduces HEK cells and ACE2-HEK cells with similar efficiency, while the transduction of Spike (SARS-CoV-2, BA.2) pseudotyped VSV Delta G depends on ACE2 expression.

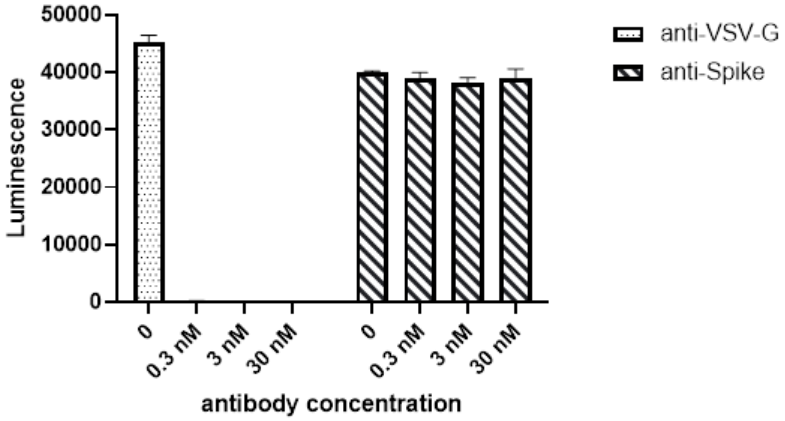

Figure 2. Neutralization assay.
Approximately 20,000 cells/well of Vero-E6 cells were seeded in a 96-well white clear-bottom assay plate. The next day, cells were infected with VSV-G pseudotyped VSV Delta G (Luciferase reporter) pre-mixed with anti-VSV-G (clone 8G5F11, Millipore Sigma# MABF2337) or anti-spike (Clone G10xA1, BPS Bioscience #101326). After 18 hours of transduction, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure luciferase activity. The transduction of VSV-G pseudotyped VSV Delta G is inhibited by anti-VSV-G antibody, not by the anti-spike antibody.

References

Whitt MA. "Generation of VSV pseudotypes using recombinant ΔG-VSV for studies on virus entry, identification of entry inhibitors, and immune responses to vaccines." *Journal of virological methods* (2010) **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
Spike (SARS-CoV-2) (BA.2, Omicron Variant) Pseudotyped VSV Delta G (Luciferase Reporter)	78635	500 µl x 2
Spike (BA.2, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luciferase Reporter)	78625	500 µl x 2