

## Description

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. 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 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. Among them, BA.4 and BA.5 have identical mutations on their spike protein. The spike protein of BA.4 and BA.5 are referred as BA.4/5 in this datasheet.

The Spike (BA.4/5, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentiviruses were produced with SARS-CoV-2 Spike (Genbank Accession #QHD43416.1 containing all the Omicron BA.4/5 mutations; see below for details) as the envelope glycoprotein instead of the commonly used VSV-G. These pseudovirions contain the firefly luciferase gene driven by a CMV promoter (Figure 1), therefore, the spike-mediated cell entry can be measured via luciferase activity. The Spike (BA.4/5, Omicron Variant) (SARS-CoV-2) pseudovirus can be used to measure the activity of a neutralizing antibody against SARS-CoV-2 Omicron BA.4/5 variant in a Biosafety Level 2 facility.

As shown in Figures 2 and 3, the Spike Omicron BA.4/5 pseudovirus has been validated for use with target cells ACE2-HEK293 (which overexpress ACE2; BPS Bioscience #79951).

## Spike Mutations in BA.4/5, Omicron Variant:

Del69-70, T19I, LPPA24-27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K

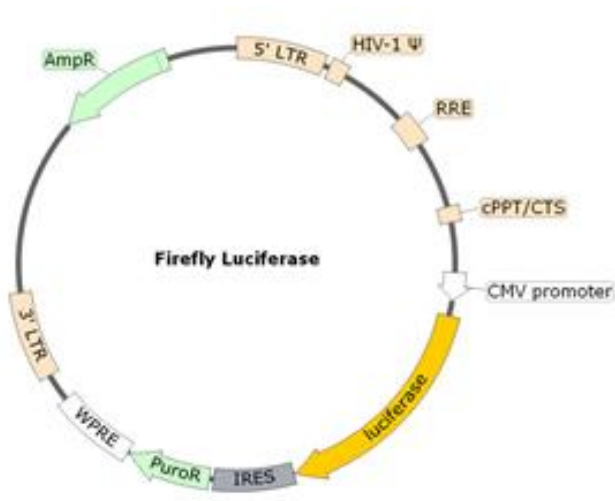


Figure 1. Schematic of the Luciferase Reporter in SARS-CoV-2 Spike Pseudovirion

## Application

Screen for or titrate neutralizing antibodies against SARS-CoV-2 Spike Omicron BA.4/5 variant in ACE2-HEK293 cells.

### Formulation

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

### Supplied as

The titer will vary with each lot; the exact value is provided with each shipment. Based on experiments performed by scientists at BPS Bioscience, 78651-1 (100 µl) provides sufficient signal-to-noise ratio to perform 100 reactions, and 78651-2 (500 µl x2) for 1000 reactions. The amount of virus added to the cells can even 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 Required 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	<a href="#">BPS Bioscience #60187</a>
ACE2-HEK293 Recombinant Cell Line	<a href="#">BPS Bioscience #79951</a>
Spike Neutralizing Antibody (Clone G10xA1) (SARS-CoV-2)	<a href="#">BPS Bioscience #101326</a>
ONE-Step™ Luciferase assay system	<a href="#">BPS Bioscience #60690</a>
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 gene can be measured approximately 48-72 hours after transduction.

The luminescence reading can be influenced by multiple-factors including cells, detection reagent or luminometer. To maximize the use of the virus, a pre-test can be carried out to determine the 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 into white, clear-bottom, 96-well microplate in 90  $\mu$ l of Thaw Medium 1 (BPS Bioscience #60187) (This step can be done during incubation of the antibody with Spike pseudotyped lentivirus).
2. Thaw the pseudovirus at room temperature. Dilute the pseudovirus with Thaw Medium 1 according to your pretest results.
3. Prepare serial dilutions of anti-Spike or anti-ACE2 antibody in Thaw Medium 1.
  - a. To test an anti-Spike antibody, preincubate 5  $\mu$ l of diluted SARS-CoV-2 Spike pseudotyped lentivirus with 5  $\mu$ l of diluted anti-Spike antibody for 30 minutes. After incubation, add 10  $\mu$ l of virus/antibody mix into each well of the ACE2-HEK293 cells.
  - b. To test an anti-ACE2 antibody, add 5  $\mu$ l of diluted anti-ACE2 antibody to the ACE2-HEK293 cells and incubate for 30 minutes. Add medium only to the “no-antibody” positive controls. At the end of the incubation, add 5  $\mu$ l of diluted SARS-CoV-2 Spike pseudotyped lentivirus into each well.
4. For control wells, the same number of ACE2-HEK293 cells were seeded, but no virus or antibody was added.
5. Incubate the plates at 37°C with 5% CO<sub>2</sub>.

**Day 3:**

1. Approximately 48-66 hours after transduction, prepare the ONE-Step™ Luciferase reagent per the recommended protocol. Add 100  $\mu$ l 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.

Figures and Validation Data

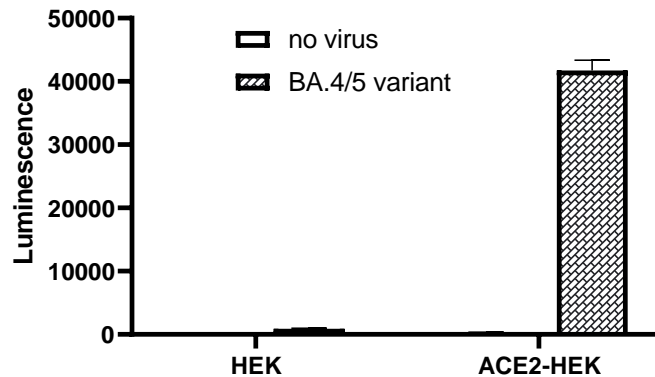


Figure 2. Transduction of ACE2-HEK293 cells. Approximately 8,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5  $\mu$ L (prediluted 5-fold) of Spike (BA.4/5, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc reporter). After 66 hours of transduction, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to cells to measure the luciferase activity. The Spike Pseudotyped Lentivirus transduced ACE2-HEK293 with much greater efficiency compared with HEK293 parental cells, indicating the transduction is dependent upon ACE2 expression.

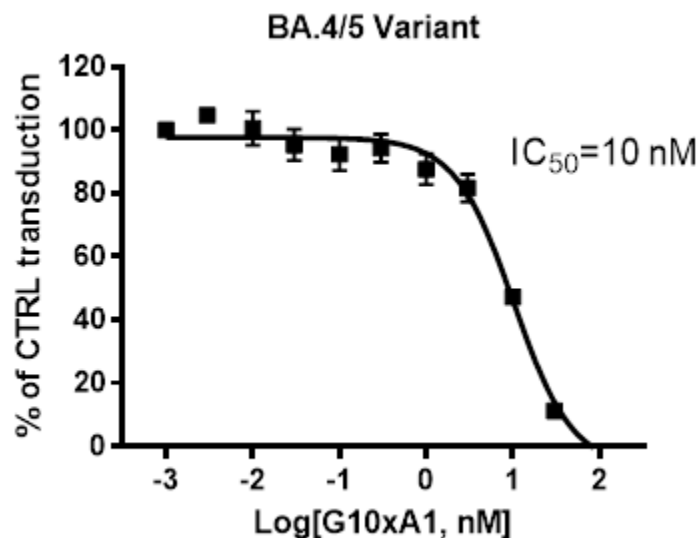


Figure 3. Neutralization assay using anti-SARS-CoV-2 Spike antibody. Approximately 8,000 ACE2-HEK293 cells/well were transduced with Spike (BA.4/5, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc reporter)/anti-Spike antibody mix. After 66 hours of transduction, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to cells to measure the luciferase activity. The transduction efficiency was determined by measuring the 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.

## Troubleshooting Guide

Visit [bpsbioscience.com/lentivirus-faq](https://bpsbioscience.com/lentivirus-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

## Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x 2
ACE2 - HEK293 Recombinant Cell Line	79951	2 vials
Spike Variants (SARS-CoV-2) Pseudotyped Lentivirus Pack (Luciferase Reporter)	78616	12 x 100 µl
Spike (BA.4/5, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78646	500 µl x 2
Spike (B.1.617 Variant) Pseudotyped Lentivirus (Luc Reporter)	78204	500 µl x 2
Spike (B.1.617.1, Kappa Variant) Pseudotyped Lentivirus (Luc Reporter)	78205	500 µl x 2
Spike (B.1.618 Variant) Pseudotyped Lentivirus (Luc Reporter)	78206	500 µl x 2
Spike (B.1.1.7, Alpha Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78112	500 µl x 2
Spike (B.1.429, Epsilon Variant) Pseudotyped Lentivirus (Luc Reporter)	78172	500 µl x 2
Spike (B.1.351, Beta Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78142	500 µl x 2
Spike (B.1.617.2; Delta Variant) Pseudotyped Lentivirus (Luc Reporter)	78215	500 µl x 2
Spike (B.1.617.2; Delta Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78216	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
Spike (BA.2.12.1, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78645	500 µl x 2
Spike (BA.2.12.1, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78646	500 µl x 2