

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

As shown in Figure 2, 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 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

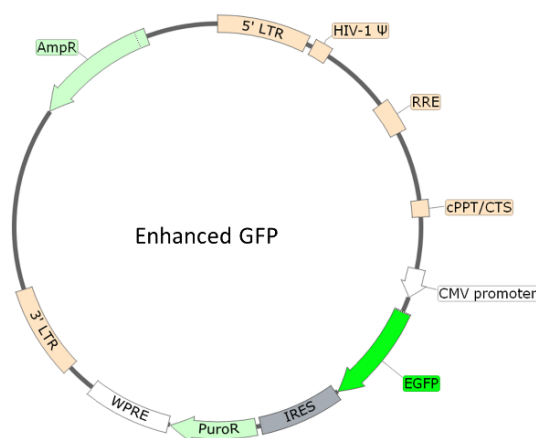


Figure 1. Schematic of the eGFP Reporter in Spike (BA.4/5, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus

## Applications

Screen neutralizing antibodies against the SARS-CoV-2 BA.4/5 variant in ACE2-HEK293 cells

## Formulation

The lentiviruses were produced from HEK293T cells. Supplied in medium containing 90% DMEM + 10% FBS.

### Titer

The titer will vary with each lot; the exact value is provided with each shipment.

### Storage



Lentiviruses are 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



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. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS 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 designed protocol. BPS Bioscience media and reagents 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>
96-well white clear-bottom assay plate	Corning #3610

### Assay Protocol

The following protocol is a general guideline for transducing ACE2-HEK293 cells using Spike (BA.4/5, Omicron Variant) (SARS-CoV-2) pseudotyped lentivirus (eGFP 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.

#### Day 1:

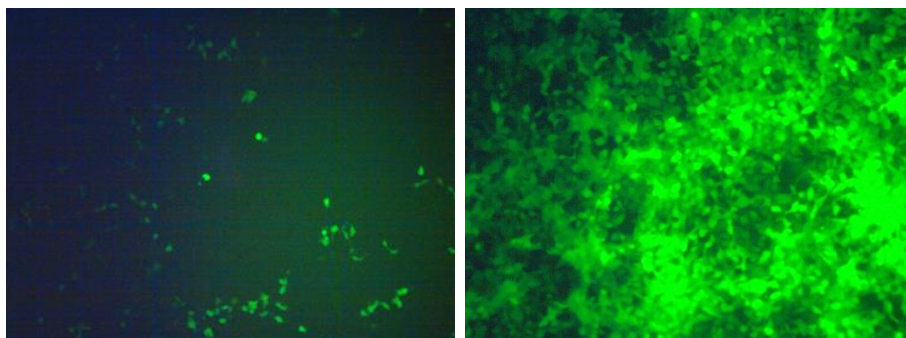
1. Plate ACE2-HEK293 cells at a density of 5,000-10,000 cells per well into white clear-bottom 96-well microplate in 50 µl of Thaw Medium 1 (BPS Bioscience #60187).
2. Add 1-5 µl of Spike (BA.4/5, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP reporter) into each well.

Optional: Add polybrene to each well at a final concentration of 5 µg/ml.

3. Incubate the plates at 37°C with 5% CO<sub>2</sub>.

**Day 3:**

1. Approximately 48-72 hours after transduction, examine the expression of eGFP in the target cells by fluorescence microscopy.



*Figure 2. Transduction of ACE2-HEK293 cells using Spike (BA.4/5, Omicron Variant) Pseudotyped Lentivirus (eGFP Reporter). Approximately 5,000 cells/well of ACE2-HEK293 cells (right) or HEK293 parental cells (left) were seeded and transduced on the same day with 5  $\mu$ l/well of Spike (BA.4/5, Omicron variant) pseudotyped lentivirus (eGFP reporter). After 66 hours of transduction, the expression of eGFP in the target cells was observed under a fluorescence microscope.*

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**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).

SPIKE (BA.4/5,OMICRON VARIANT) (SARS-CoV-2)  
PSEUDOTYPED LENTIVIRUS (eGFP REPORTER)

**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 (BA.2, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78626	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.1.351, Beta Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78160	500 µl x 2
Spike (P.1, Gamma Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78159	500 µl x 2
Spike (B.1.1.7, Alpha Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78158	500 µl x 2
Spike (B.1.617.2.1; Delta Plus Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78219	500 µl x 2
Spike (D614G) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78035	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 (eGFP Reporter)	78349	500 µl x 2
Spike (BA.1.1, Omicron Variant R346K) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78624	500 µl x 2
Spike (BA.2.12.1, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78645	500 µl x 2