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Zuschläge

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- Trockeneiszuschlag
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- Expressversand

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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 and ACE2 may offer protection against the viral infection.

In Brazil, a variant called B.1.1.28.1 (P.1) was first identified in the fall of 2020. This variant has many mutations which may lead to higher transmissibility and infectivity. Among these mutations, three (K417T, E484K, N501Y) appear to be crucial. The Spike (K417T, E484K, N501Y) (SARS-CoV-2) Pseudotyped Lentiviruses were produced with SARS-CoV-2 Variant Spike (Genbank Accession #QHD43416.1 with mutations K417T, E484K, and N501Y) as the envelope glycoproteins 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 (SARS-CoV-2, K417T, E484K, N501Y) pseudotyped lentivirus can be used to measure the activity of neutralizing antibody against SARS-CoV-2 K417T, E484K, N501Y variant in intact cells using a Biosafety Level 2 facility.

Application

1. Study the mechanism of viral transduction of SARS-CoV-2 K417T, E484K, N501Y variant.
2. Screening for neutralizing antibodies that inhibit the binding of SARS-CoV-2 Spike (K417T, E484K, N501Y variant) to ACE2.

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.

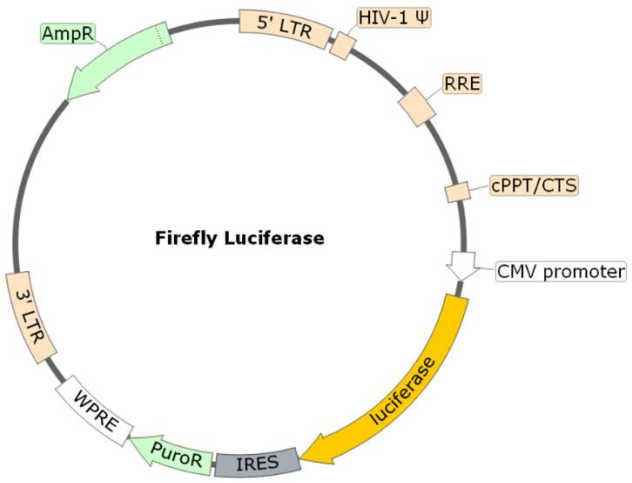

Figure 1. Schematic of the Luciferase Reporter in Spike (SARS-CoV-2, K417T, E484K, N501Y) Pseudotyped Lentivirus

Materials Required but Not Supplied

These materials are not supplied with this lentivirus but are necessary to follow the designed protocol. BPS Bioscience media, reagents, and luciferase assay systems 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
Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luc reporter)	BPS Bioscience, #79942
ACE2- HEK293 Recombinant Cell Line	BPS Bioscience, #79951
Anti-SARS-CoV-2 Spike Neutralizing Antibody	BPS Bioscience, #100793
96-well white clear-bottom assay plate	Corning, #3610
ONE-STEP Luciferase Assay System	BPS Bioscience, #60690

Assay Protocol

The following protocol is a general guideline for transducing ACE2-HEK293 cells using Spike (SARS-CoV-2, K417T, E484K, N501Y) 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.

1. Day 1: Harvest ACE2-HEK293 cells from culture and seed cells at a density of 5,000-10,000 cells per well into white opaque 96-well microplate in 50 µl of Thaw Medium 1 (BPS Bioscience, #60187). Incubate cells at 37°C with 5% CO_2 overnight.

   To demonstrate transduction is dependent on ACE2, the same number of HEK293 parental cells can be seeded in Thaw Medium 1 as control cells.

2. Day 2: prepare serial dilutions of anti-Spike or anti-ACE2 antibody in Thaw Medium 1.

   To test anti-Spike antibody, preincubate 5 µl of the Spike (SARS-CoV-2, K417T, E484K, N501Y) pseudotyped lentivirus with 5 µl of diluted anti-Spike antibody for 30 minutes. After incubation, add 10 µl of virus/antibody mix into each well of the ACE2-HEK293 cells.

   To test anti-ACE2 antibody, add 5 µl of diluted anti-ACE2 antibody into each well of ACE2-HEK293 cells and incubate for 30 minutes. At the end of the incubation, add 5 µl of Spike (SARS-CoV-2, K417T, E484K, N501Y) pseudotyped lentivirus into each well.

   For control wells, the same number of ACE2-HEK293 cells are seeded, but no virus or antibody are added.

   Incubate the plates at 37°C with 5% CO_2 overnight.

   Alternatively, seeding cells and the transduction can be performed on the same day.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 50 µl of fresh Thaw Medium 1 to each well.

   If the test antibody does not adversely affect the target cells, it is not necessary to change the medium on Day 3.

4. Day 4, approximately 48-60 hours after transduction, prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 50 µl of ONE-Step™ Luciferase Assay reagent per well. 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.

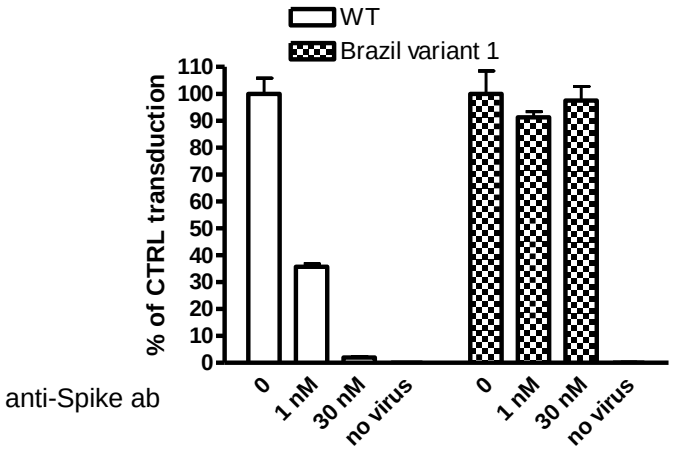

Figure 2. Transduction of ACE2-HEK293 Cells using Spike (SARS-CoV-2, K417T, E484K, N501Y) Pseudotyped Lentivirus.
Approximately 8,000 ACE2-HEK293 cells/well were transduced with 10 µl/well of either wild type Spike (SARS-CoV-2) pseudotyped lentivirus (Luc reporter) (BPS Bioscience, #79942) or Spike (SARS-CoV-2, K417T, E484K, N501Y) pseudotyped lentivirus (Luc reporter) (BPS Bioscience, #78143) mixed with anti-Spike antibody (BPS Bioscience, #100793; clone#414-1). After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience, #60690) was added to cells to measure the luciferase activity. The anti-Spike antibody inhibits the transduction of the wild-type Spike (SARS-CoV-2) pseudotyped lentivirus, but not the K417T, E484K, N501Y variant.

License Disclosure
Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide
Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	79942	500 µl x2
Spike (SARS-CoV-2, B.1.1.7 variant) Pseudotyped Lentivirus (Luc Reporter)	78112	500 µl x2
Spike (SARS-CoV-2, B.1.351 variant) Pseudotyped Lentivirus (Luc Reporter)	78142	500 µl x2
Spike (SARS-CoV-2, P.1) Pseudotyped Lentivirus (Luc Reporter)	78144	500 µl x2
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x2
Spike Pseudotyped Lentivirus (eGFP Reporter)	79981	500 µl x2
Bald Lentiviral Pseudoviron (eGFP Reporter)	79987	500 µl x2
Spike Pseudotyped Lentivirus (Luciferase-eGFP Dual Reporter)	79982	500 µl x2
Bald Lentiviral Pseudoviron (Luciferase-eGFP Dual Reporter)	79988	500 µl x2
ACE2-HEK293 Recombinant Cell Line	79951	2 vials