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Produktinformation

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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- 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 of SARS-CoV-2 and ACE2 may offer protection against the viral infection. A variant called B.1.621 (also known as the Mu Variant) was first identified in Columbia in early 2021. This variant has a number of mutations that may increase morbidity and mortality and allow the virus to spread more easily and quickly than other variants.

The Spike (B.1.621, Mu Variant) (SARS-CoV-2) Pseudotyped Lentiviruses were produced with SARS-CoV-2 B.1.621 Variant Spike (Genbank Accession #QHD43416.1 with B.1.621 mutations; see below for details) 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 (B.1.621, Mu Variant) (SARS-CoV-2) pseudotyped lentivirus can be used to measure the activity of neutralizing antibody against the B.1.621 variant in a Biosafety Level 2 facility.

Spike Mutations in B.1.621 Variant:

T95I
Y144S
Y145N
R346K
E484K
N501Y
D614G
P681H
D950N

Applications

1. Study the mechanism of viral transduction
2. Screening for neutralizing antibodies that inhibit the interaction between ACE2 and the Spike protein of SARS-CoV-2 Mu variant.

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 or HEK293 Cell Culture Medium	BPS Bioscience, #60187
ACE2-HEK293 Recombinant Cell Line	BPS Bioscience, #79951
Spike S1 Neutralizing Antibody (Clone C-A11) (SARS-CoV-2)	BPS Bioscience, #101024
96-well tissue culture treated, white clear-bottom assay plate	Corning, #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience, #60690

Media Formulation

Thaw Medium 1 (BPS Bioscience, #60187):
MEM medium (Hyclone, #SH30024.01) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Hyclone, #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

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.

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, clear-bottom, 96-well microplate in 90 µl of Thaw Medium 1 (BPS Bioscience, #60187) (This step can be done during incubation of antibody and Spike pseudotyped lentivirus).

   Prepare serial dilutions of anti-Spike or anti-ACE2 antibody in Thaw Medium 1.

   To test anti-Spike antibody, preincubate 5 µl of the SARS-CoV-2 Spike 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 SARS-CoV-2 Spike pseudotyped lentivirus into each well.

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

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

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

2. Day 3:

Approximately 48-60 hours after transduction, prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 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.

Figures and Validation Data

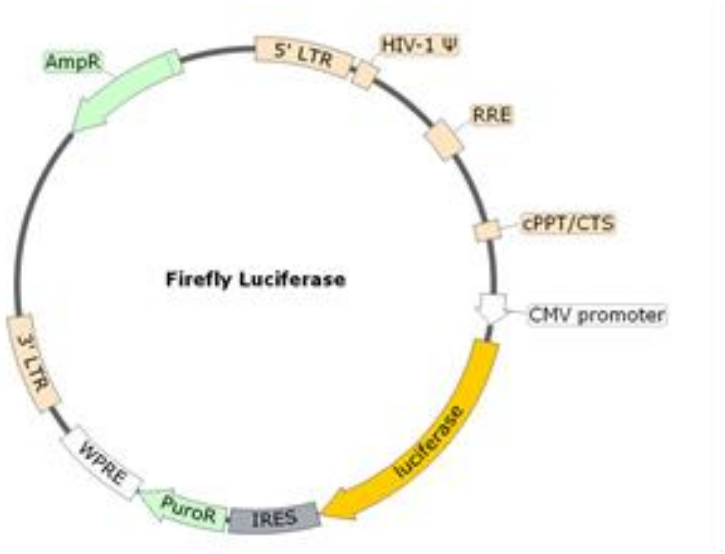

Figure 1. Schematic of the Luciferase Reporter in SARS-CoV-2 Spike (B.1.621 Variant) Pseudovirion

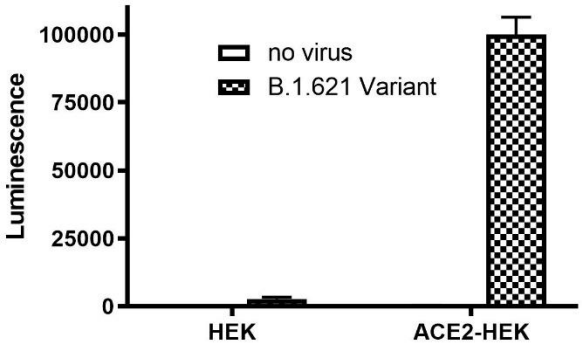

Figure 2. Transduction of ACE2-HEK293 cells
Approximately 8,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5 µl/well of Spike (B.1.621, Mu Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc reporter). After 48 hours of transduction, ONE-Step™ Luciferase reagent (BPS Bioscience, #60690) was added to cells to measure the luciferase activity. The Spike (B.1.621 Mu Variant) (SARS-CoV-2) Pseudotyped Lentivirus transduced ACE2-HEK293 with much greater efficiency compared with HEK293 parental cells, indicating the transduction is dependent upon ACE2 expression.

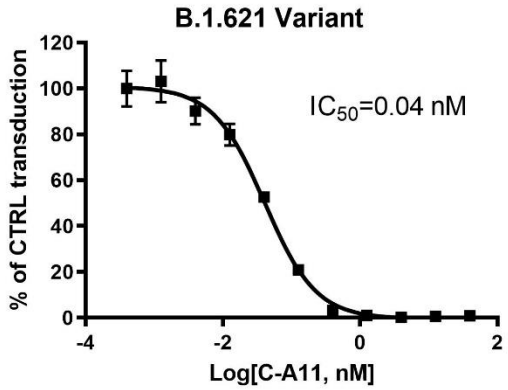

Figure 3: Neutralization assay by anti-SARS-CoV-2 Spike antibody
Approximately 8,000 ACE2-HEK293 cells/well were transduced with 10 µl/well of Spike (B.1.621, Mu Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc reporter)/anti-Spike antibody mix. After 48 hours of transduction, ONE-Step™ Luciferase reagent (BPS Bioscience, #60690) was added to cells to measure 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 S1 Neutralizing Antibody (SARS-CoV-2) (Clone: C-A11) (BPS Bioscience, #101024) is shown.

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

Related Products

Products	*Catalog #*	*Size*
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x 2
ACE2 - HEK293 Recombinant Cell Line	79951	2 vials
Spike (B.1.617.2; Delta Variant) Pseudotyped Lentivirus (Luc Reporter)	78215	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) Pseudotyped lentivirus (Luc Reporter)	78112	500 µl x 2
Spike (P.1; Gamma Variant) Pseudotyped Lentivirus (Luc Reporter)	78144	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) Pseudotyped lentivirus (Luc Reporter)	78142	500 µl x 2
Spike (B.1.617.2; Delta Variant) Pseudotyped Lentivirus (eGFP Reporter)	78216	500 µl x 2
Spike (B.1.351; Beta Variant) Pseudotyped Lentivirus (eGFP Reporter)	78160	500 µl x 2
Spike (B.1.1.7; Alpha Variant) Pseudotyped Lentivirus (eGFP Reporter)	78158	500 µl x 2