SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

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.1.1.529 BA.1 (also known as the 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. A sub-lineage of BA.1 with an R346K substitution in the spike protein is classified as B.1.1.529 BA.1.1.

The Spike (B.1.1.529 BA.1.1, Omicron Variant R346K) (SARS-CoV-2) Pseudotyped Lentiviruses were produced with SARS-CoV-2 B.1.1.529 BA.1.1 Variant Spike (Genbank Accession #QHD43416.1 with B.1.1.529 BA.1.1 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 (B.1.1.529 BA.1.1, Omicron Variant R346K Variant) (SARS-CoV-2) pseudovirus can be used to measure the activity of a neutralizing antibody against SARS-CoV-2 B.1.1.529 BA.1.1 variant in a Biosafety Level 2 facility.

As shown in Figures 2 and 3, the Spike B.1.1.529 BA.1.1 pseudovirus has been validated for use with target cells ACE2-HEK293 (which overexpress ACE2; BPS Bioscience #79951).

Spike Mutations in B.1.1.529 BA.1.1 Omicron Variant R346K:

A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, R346K, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F

Application

Screen for or titrate neutralizing antibodies against SARS-CoV-2 Spike B.1.1.529 BA.1.1 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, 78623-1 (100 µl) provides sufficient signal-to-noise ratio to perform 100 reactions, and 78623-2 (500 µl x2) for 1000 reactions. The amount of virus added to the cells can even be titrated 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	BPS Bioscience #60187
ACE2-HEK293 Recombinant Cell Line	BPS Bioscience #79951
Spike Neutralizing Antibody (Clone G10xA1) (SARS-CoV-2)	BPS Bioscience #101326
Spike Neutralizing Antibody (Clone G10xA5) (SARS-CoV-2)	BPS Bioscience #101327
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 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 DMEM high glucose medium + 10% FBS. 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 µl 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 DMEM high glucose medium + 10% FBS according to your pretest results.

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

   To test an anti-Spike antibody, preincubate 5 µl of diluted 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 an anti-ACE2 antibody, add 5 µl 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 µl 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.
   Incubate the plates at 37°C with 5% CO2.

Day 3:

Approximately 48-66 hours after transduction, prepare the ONE-Step™ Luciferase reagent per the 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

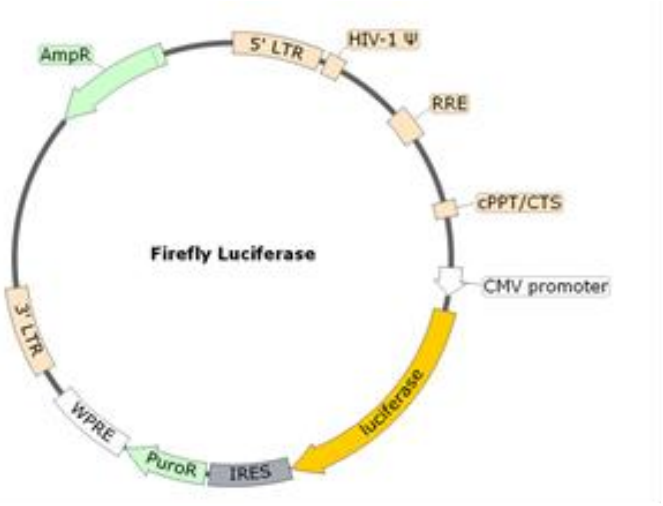

Figure 1. Schematic of the Luciferase Reporter in SARS-CoV-2 Spike Pseudovirion

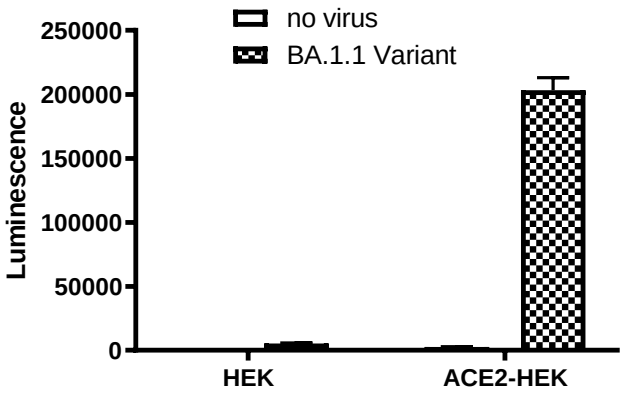

Figure 2. Transduction of ACE2-HEK293 cells. Approximately 8,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5 µL (prediluted 5-fold) of Spike (B.1.1.529 BA.1.1, Omicron Variant R346K) (SARS-CoV-2) Pseudotyped Lentivirus (Luc reporter). After 66 hours of transduction, 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.

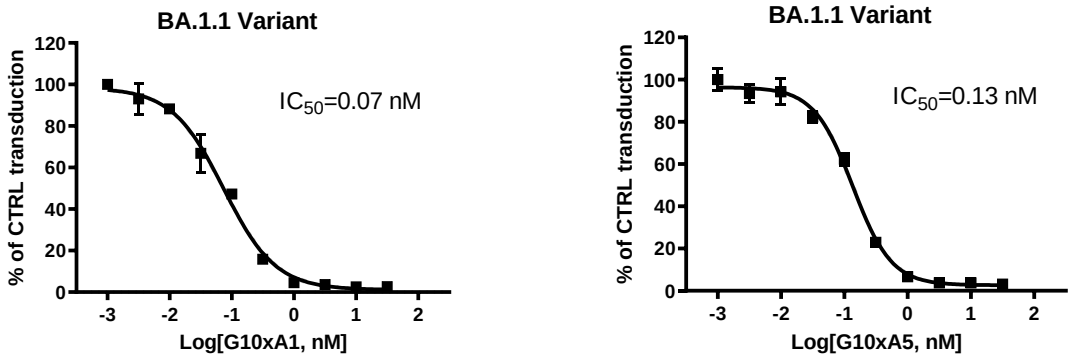

Figure 3. Neutralization assay using anti-SARS-CoV-2 Spike antibody. Approximately 8,000 ACE2-HEK293 cells/well were transduced with Spike (B.1.1.529 BA.1.1, Omicron Variant R346K) (SARS-CoV-2) Pseudotyped Lentivirus (Luc reporter)/anti-Spike antibody mix. After 66 hours of transduction, ONE-Step™ Luciferase 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 curves for Spike Neutralizing Antibody (SARS-CoV-2) A) Clone G10xA1 (BPS Bioscience #101326) and B) Clone G10xA5 (BPS Bioscience #101327) are shown.

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 Variants (SARS-CoV-2) Pseudotyped Lentivirus Pack (Luciferase Reporter)	78616	12 x 100 µl
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) (SARS-CoV-2) 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 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78348	500 µl x 2
Spike (B.1.1.529 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (eGFP Reporter)	78349	500 µl x 2