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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

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

Spike(SARS-CoV-2) Pseudotyped Lentivirus (Luc-eGFP Dual Reporter)

Catalog#: 79982

Product 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 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.

The SARS-CoV-2 Spike Pseudotyped Lentivirus (Luc-eGFP dual reporter) were produced by replacing the VSV-G fusion glycoprotein with SARS-CoV-2 Spike protein (Genbank Accession #QHD43416.1) as a surrogate viral envelope protein.. These pseudovirions also contain a firefly luciferase and eGFP cassette (Luc-P2A-eGFP) driven by a CMV promoter (Figure 1). The luciferase and eGFP are coexpressed under the CMV promoter in the transduced cells. Therefore, the Spike-mediated entry into the target cell can be conveniently measured via luciferase reporter activity or eGFP expression. The SARS-CoV-2 Spike pseudotyped lentivirus can be used in a cellular assay to measure the activity of neutralizing antibody against SARS-CoV-2.

Application

1. Study the mechanism of viral transduction.
2. Screening for neutralizing antibodies for SARS-CoV-2 Spike and ACE2.

Formulation

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

Titer

The functional titer of SARS-CoV-2 Spike Pseudotyped Lentivirus (~1-2 x 10^5 TU/ml) is significantly lower than VSV-G pseudotyped lentivirus (>10^7 TU/ml), although the amount of lenti particles are similar (>10^9 LP/ml). The exact titer 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.

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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.

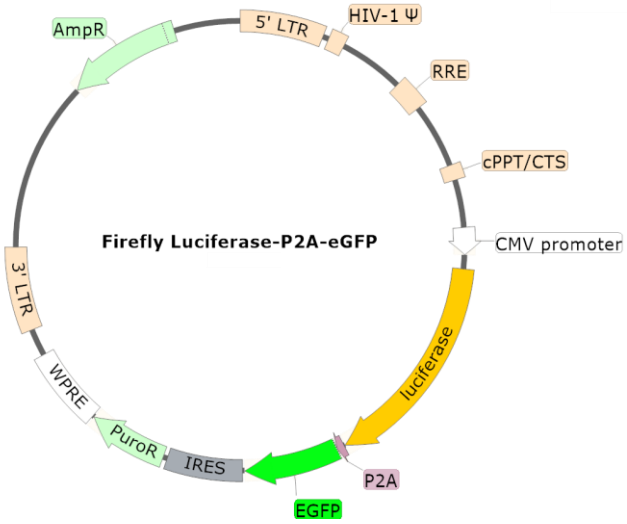

Figure 1. Schematic of the Luciferase-P2A-eGFP Reporter in SARS-CoV-2 Spike Pseudovirion

Materials Required but Not Supplied

- HEK293 growth medium or use Thaw Medium 1 (BPS Bioscience #60187): MEM with 10% FBS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 1% Penicillin/Streptomycin (Hyclone #SV30010.01).
- ACE2-HEK293 Recombinant Cell Line (BPS Bioscience, #79951)
- Bald lentiviral pseudovirion (Luc-eGFP dual reporter) (BPS Bioscience, #79988)
- Neutralizing Anti-SARS-CoV-2 Spike antibody (clone 414-1, BPS Bioscience, #100793 or clone 14-2, BPS Bioscience, #100792)
- 96-well tissue culture treated, white clear-bottom assay plate (Corning, #3610)
- ONE-Step™ luciferase assay system (BPS Bioscience, #60690)

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Assay Protocol

The following protocol is a general guideline for transducing ACE2-HEK293 cells using SARS-CoV-2 Spike pseudotyped lentivirus (Luc-eGFP dual 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.

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 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 were seeded, but no virus or antibody was added.

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

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 tested 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.

5. To check the expression of eGFP: on Day 4, approximately 48-60 hours after transduction, examine cells using fluorescence microscopy or analyze by flow cytometry. MOI should be optimized based on the number of eGFP positive cells.

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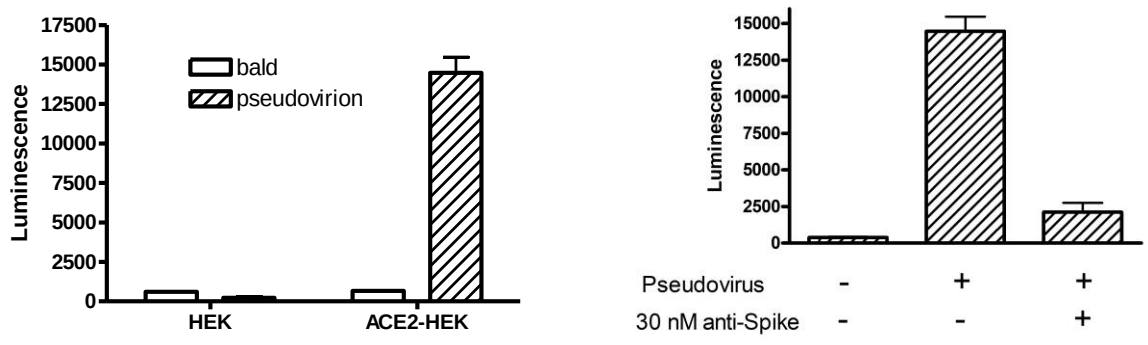

A B

Figure 2. Transduction of ACE2-HEK293 Cells Monitored by Luciferase Activity.
A. Approximately 10,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luc-eGFP dual reporter). After 18 hours of transduction, the medium was changed to fresh HEK growth medium (Thaw Medium 1). After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience, #60690) was added to cells to measure the luciferase activity. The SARS-CoV-2 Spike pseudotyped lentivirus transduced ACE2-HEK293 cells with much greater efficiency compared with HEK293 parental cells, indicating the transduction is dependent upon ACE2 expression. The bald lentiviral pseudovirion (BPS Bioscience #79988), where no envelope glycoprotein is expressed, was used as a negative control.

B. Approximately 10,000 cells/well of ACE2-HEK293 cells were transduced with 5 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luc-eGFP dual reporter) mixed with 5 µl/well of anti-Spike antibody (BPS Bioscience, #100793). After 18 hours of transduction, the medium was changed to fresh HEK growth medium (Thaw Medium 1). After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience, #60690) was added to cells to measure the luciferase activity.

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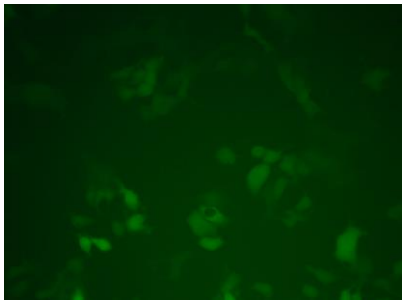

Figure 3. Transduction of ACE2-HEK293 Cells Monitored by eGFP Expression. Approximately 10,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 20 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luc-eGFP dual reporter) or bald lentiviral pseudovirion (Luc-eGFP dual reporter), BPS Bioscience #79988. After 18 hours of transduction, the medium was changed to fresh HEK293 growth medium (Thaw Medium 1). After 66 hours of transduction, the expression of eGFP in the target cells was examined using a fluorescence microscope.

As negative controls, almost no eGFP expression was observed in ACE2-HEK cells transduced with Bald Lentiviral Pseudovirion (Luc-eGFP dual reporter) or HEK parental cells transduced with SARS-CoV-2-Spike pseudotyped lentivirus (Luc-eGFP dual reporter), indicating the transduction is dependent upon the ACE2 receptor.

Related Products

Product	Cat. #	Size
SARS-CoV-2 Spike Pseudotyped Lentivirus (Luciferase Reporter)	79942	500 µl x2
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x2
SARS-CoV-2 Spike Pseudotyped Lentivirus (eGFP Reporter)	79981	500 µl x2
Bald Lentiviral Pseudoviron (eGFP Reporter)	79987	500 µl x2
Bald Lentiviral Pseudoviron (Luciferase-eGFP dual Reporter)	79988	500 µl x2
Spike S1 Neutralizing Antibody (SARS-CoV-2) (Clone: 414-1)	100793	100 µg
Spike S1 Neutralizing Antibody (SARS-CoV-2) (Clone: 414-2)	100792	100 µg
eGFP Lentivirus	79979	500 µl x2
Firefly Luciferase-eGFP Lentivirus	79980	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (G418)	79692-G	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Hygromycin)	79692-H	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Puromycin)	79692-P	500 µl x2