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Produktinformation

- Forschungsprodukte & Biochemikalien
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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

Severe acute respiratory syndrome (SARS) was the first new infectious disease identified in the twenty-first century. It is a viral respiratory disease caused by severe acute respiratory syndrome coronavirus (SARS-CoV-1). The first known cases occurred in November 2002, and the syndrome caused the 2002–2004 SARS outbreak. Since 2004, no cases of SARS-CoV-1 have been reported worldwide. A virus very similar to SARS-CoV-1 was discovered in late 2019. This virus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is the causative pathogen of COVID-19, the spread of which started the COVID-19 pandemic.

SARS-CoV-1 attaches to the host cell surface before entering the cell. The Spike protein on the virus recognizes and binds to the Angiotensin-Converting Enzyme 2 (ACE2) receptor found on the surface of human airway epithelia as well as lung parenchyma. Drugs targeting the interaction between the Spike protein of SARS-CoV-1 and ACE2 may offer protection against the viral infection.

The Spike (SARS-CoV-1) Pseudotyped Lentiviruses were produced with SARS-CoV-1 Spike (Genbank Accession #YP_009825051.1) 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 (SARS-CoV-1) pseudovirus can be used to measure the activity of a neutralizing antibody against SARS-CoV-1 in a cellular context, using a Biosafety Level 2 facility.

As shown in Figure 2, the Spike (SARS-CoV-1) Pseudotyped Lentiviruses has been validated for use with target cells ACE2-HEK293 (which overexpress ACE2; BPS Bioscience #79951).

Application

Screen for or titrate neutralizing antibodies against SARS-CoV-1 Spike protein

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, 78614-1 (100 µl) provides sufficient signal-to-noise ratio to perform 100 reactions, and 78614-2 (500 µl x2) is sufficient for 1000 reactions. The amount of virus added to the cells can be titrated even further, 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
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-1 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 the 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-1 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-1 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% CO_2.

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. "Blank" value is subtracted from all readings. The transduction efficacy is determined by measuring the luciferase activity.

Figures and Validation Data

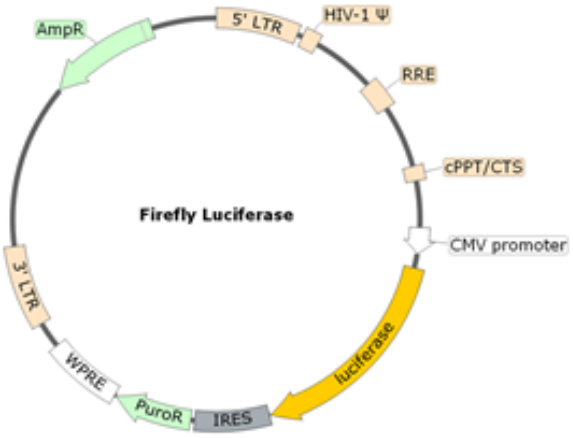

Figure 1. Schematic of the Luciferase Reporter vector in SARS-CoV-1 Spike Pseudovirion

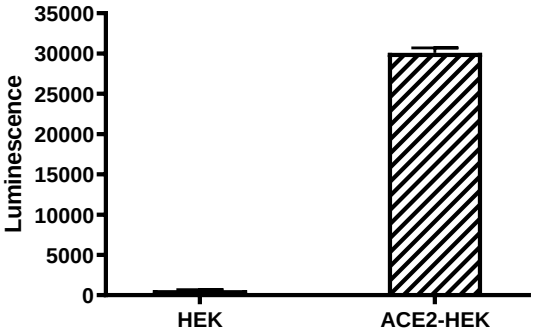

Figure 2. Transduction of ACE2-HEK293 cells. Approximately 8,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5 µL (prediluted 5-fold) of Spike (SARS-CoV-1) Pseudotyped Lentivirus (Luc reporter). After 66 hours of transduction, ONE-Step™ Luciferase reagent (BPS Bioscience #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.

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 Trimer (S1+S2), His-tag (SARS-CoV-1)	100789	100 µg
Spike S1 Neutralizing Antibody (VHH), Fc-fusion (IgG1), Avi-Tag (SARS-CoV-1)	100784	100 µg
Spike S1 Neutralizing Antibody (VHH), Fc-fusion (IgG1), Avi-Tag (SARS-CoV-1), Biotin-labeled	100785	50 µg
Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luciferase Reporter)	79942	500 µl x 2
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) 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 BA.1, Omicron Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78348	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 (Luc Reporter)	78623	500 µl x 2