REF: P30915

Covid-19 related products

- Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luciferase Reporter) -

Product Name:	SARS-CoV-2 Spike Lentiviral Particles
Catalog Number:	P30915
Reporter gene:	Luciferase
Genbank:	QHD43416.1
Format:	Reagents for 1xMW96 plate
Storage:	- 80ºC

Introduction

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. 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 were produced with SARS-CoV-2 Spike (Genbank Accession #QHD43416.1) as the envelope glycoproteins instead of the commonly used VSV-G. These pseudovirions also contain the Luciferase gene driven by a CMV promoter (Figure 1), therefore, the spike-mediated cell entry can be conveniently determined via luciferase activity.

Applications

The SARS-CoV-2 Spike pseudotyped lentivirus can be used to measure the activity of chemical compounds or neutralizing antibody against SARS-CoV-2 in a Biosafety Level 2 facility.

- Study the mechanism of viral transduction.
- Screening for chemical compounds or 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 titer will vary with each lot; the exact value is provided with each shipment.

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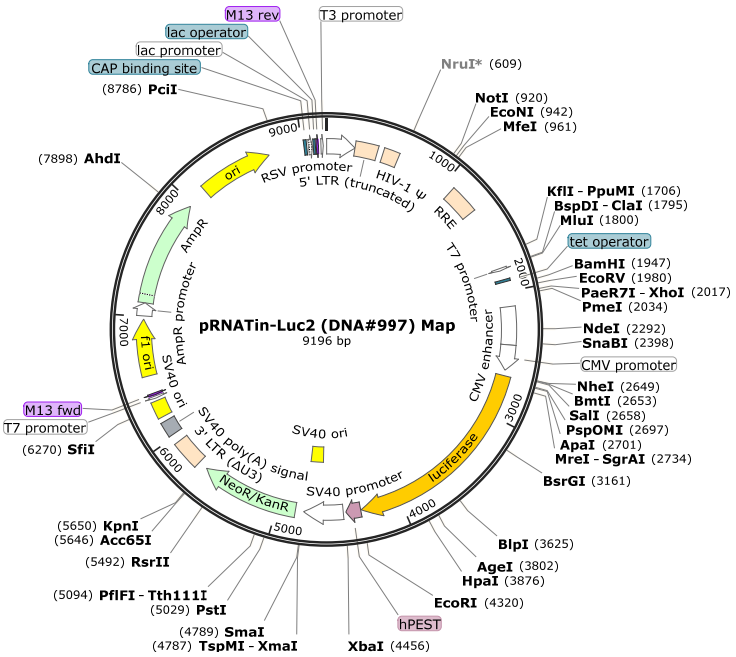

Figure 1. Schematic of the Luciferase Reporter in SARS-CoV-2 Spike Pseudovirion

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

- HEK293 growth medium (Dulbecco's Modified Eagle's Medium - high glucose (Ref: D6429 Sigma-Aldrich) supplemented with 10% FBS, 1% non- essential amino acids (Sigma Aldrich M7145), 1% Penicillin/Streptomycin (Sigma-Aldrich 516104-M).
- ACE2-HEK293 Recombinant Cell Line (Ref: P30902)
- OptiMeM (Invitrogen 31985062).
- Luciferase Assay System (Promega E150)

Assay Protocol

The following protocol is a general guideline for transducing ACE2-HEK293 cells using SARS-CoV-2 Spike pseudotyped lentivirus (Luciferase reporter) from Innoprot. 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 complete culture medium. Incubate cells at 37°C with 5% CO2 24-36h.
To demonstrate the transduction is dependent on ACE2, the same number of HEK293 parental cells are seeded in control media as control cells.

2. Day 2: Wash ACE2-HEK293 cells with OptiMem medium. Add 10 µl of SARS-CoV-2 Spike pseudotyped lentivirus (Luciferase reporter) or bald lentiviral pseudovirion (Luciferase reporter) resuspended in 90 µl of OptiMem into each well.
Incubate the plates overnight at 37°C with 5% CO_2.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 100-200 µl of fresh complete medium to each well.

4. Day 4-5, approximately 48-72 hours after transduction, the expression of luciferase in the target cells was examined using the Luciferase assay system from Promega following the manufacturer´s instructions.

Other related products

Ref: P30902 – HEK293 Cell line stably expressing ACE2
Ref: P30903 – HEK293 Cell line stably expressing green fluorescent ACE2
Ref: P30910 – HEK293T Cell Line stably expressing SARS-CoV-2 Spike Glycoprotein (D614F)
Ref: P30911 – HEK293T Cell Line stably expressing SARS-CoV-2 Spike Glycoprotein (V367F)
Ref: P30912 – HEK293T Cell Line stably expressing SARS-CoV-2 Spike Glycoprotein (V483A)
Ref: P30920 – HEK293 Cell Line stably expressing SARS-CoV-2 Nucleocapsid Protein
Ref: P30921 – HEK293 Cell Line stably expressing SARS-CoV-2 Membrane Protein

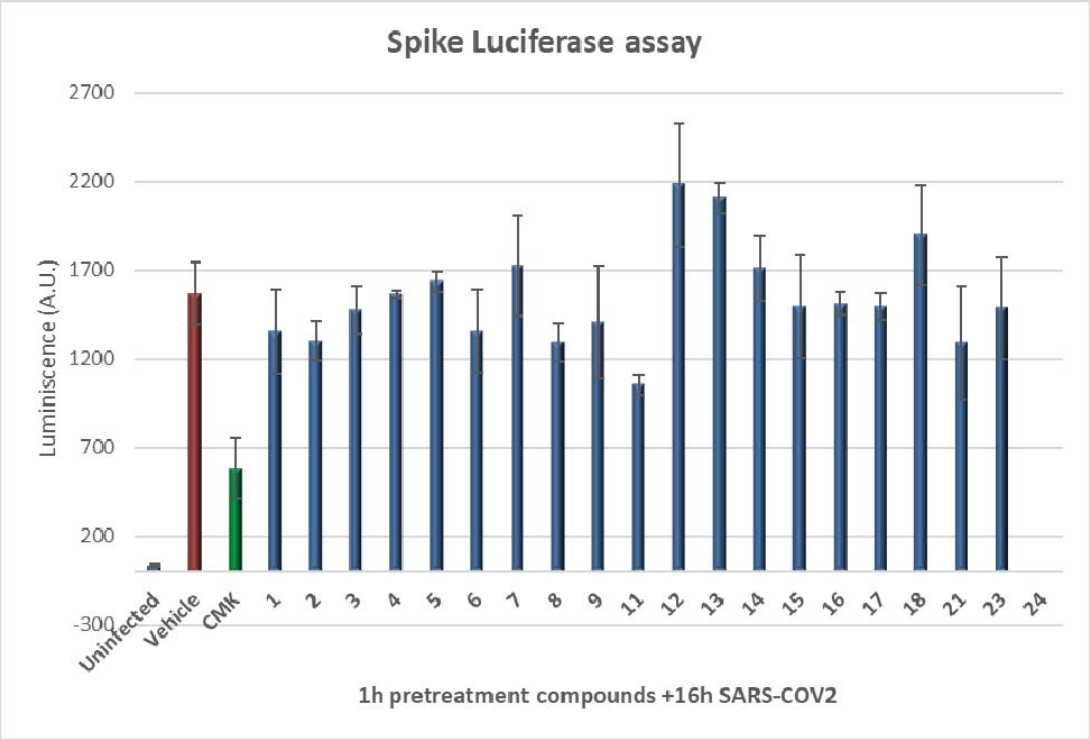

Figure 2. Compounds screening using SARS-CoV-2 Spike pseudotyped lentivirus (Luciferase reporter). Approximately 10,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 10 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luciferase reporter). After 18 hours of transduction, the medium was changed to fresh HEK293 growth medium. After 48 hours of transduction, the expression of luciferase in the target cells was observed using the Luciferase Assay system from Promega. CMK is used as inhibitor control of the pseudoparticles infection.

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