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**Data Sheet**  
***Spike (SARS-CoV-2, D614G)***  
***Pseudotyped Lentivirus (eGFP Reporter)***  
***Catalog#: 78035***

**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 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 and ACE2 may offer protection against the viral infection. A SARS-CoV-2 variant carrying the spike protein amino acid change D614G has become the most prevalent form in the global pandemic.

The SARS-CoV-2 Spike D614G Pseudotyped Lentivirus were produced with SARS-CoV-2 Spike (Genbank Accession #QHD43416.1; with D614G mutation) as the envelope glycoproteins instead of the commonly used VSV-G. These pseudovirions contain the enhanced GFP gene driven by a CMV promoter (Figure 1), therefore, the spike-mediated cell entry can be conveniently determined via eGFP activity. The SARS-CoV-2 Spike D614G pseudotyped lentivirus can be used to measure the activity of neutralizing antibody against SARS-CoV-2 in a Biosafety Level 2 facility.

**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 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

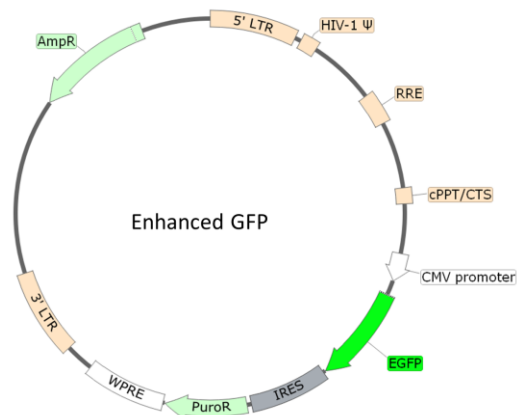
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recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.



**Figure 1. Schematic of the eGFP Reporter in SARS-CoV-2 Spike D614G Pseudovirion**

#### Materials Required but Not Supplied

- HEK293 growth medium or use **Thaw Medium 1** (BPS Bioscience #60187): MEM supplemented with 10% FBS, 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).
- ACE2-HEK293 Recombinant Cell Line (BPS Bioscience, #79951)
- Bald Lentiviral Pseudovirion (eGFP Reporter) (BPS Bioscience, #79987)

#### Assay Protocol

The following protocol is a general guideline for transducing ACE2-HEK293 cells using SARS-CoV-2 Spike D614G pseudotyped lentivirus (eGFP 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  $\mu$ l of Thaw Medium 1 (BPS Bioscience, #60187). Incubate cells at 37°C with 5% CO<sub>2</sub> overnight.

To demonstrate the transduction is dependent on ACE2, the same number of HEK293 parental cells are seeded in Thaw Medium 1 as control cells.

2. Day 2: Add 20  $\mu$ l of SARS-CoV-2 Spike D614G pseudotyped lentivirus (eGFP reporter) or bald lentiviral pseudovirion (eGFP reporter) into each well.

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

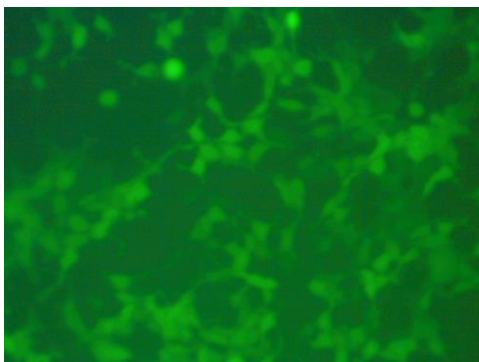
*Alternatively, seeding cells and the transduction can be performed on the same day.*

Incubate the plates overnight at 37°C with 5% CO<sub>2</sub>.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 50 µl of fresh Thaw Medium 1 to each well.

*If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 3. The target cells can be incubated with the virus for 48-72 hours before changing medium.*

4. Day 4-5, approximately 48-72 hours after transduction, the expression of eGFP in the target cells was examined using fluorescence microscopy (excitation wavelength 488 nm, emission wavelength 510 nm).



**Figure 2. Transduction of ACE2-HEK293 cells using SARS-CoV-2 Spike D614G pseudotyped lentivirus (eGFP reporter).** Approximately 8,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 20 µl/well of SARS-CoV-2-Spike D614G pseudotyped lentivirus (eGFP reporter) or bald lentiviral pseudovirion (eGFP reporter), BPS Bioscience #79987. After 66 hours of transduction, the expression of eGFP in the target cells was observed under a fluorescence microscope at  $\lambda_{exc}$  488 nm,  $\lambda_{em}$  510 nm.

As negative controls, almost no eGFP expression was observed in ACE2-HEK cells transduced with Bald Lentiviral Pseudovirion (eGFP reporter) or HEK parental cells transduced with SARS-CoV-2-Spike D614G pseudotyped lentivirus (eGFP reporter), indicating the transduction is dependent upon the ACE2 receptor.

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## Related Products

<b><u>Product</u></b>	<b><u>Cat. #</u></b>	<b><u>Size</u></b>
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x2
SARS-CoV-2 Spike Pseudotyped Lentivirus (Luciferase Reporter)	79942	500 µl x2
Bald Lentiviral Pseudovirion (eGFP Reporter)	79987	500 µl x2
SARS-CoV-2 Spike Pseudotyped Lentivirus (eGFP Reporter)	79981	500 µl x2
SARS-Cov-2 Spike D614G Pseudotyped Lentivirus (Luciferase Reporter)	78028	500 µl x2
Bald Lentiviral Pseudovirion (Luciferase-eGFP dual Reporter)	79988	500 µl x2
SARS-CoV-2 Spike Pseudotyped Lentivirus (Luciferase-eGFP Dual Reporter)	79982	500 µl x2
eGFP Lentivirus	79981	500 µl x2
Thaw Medium 1	60187	100 ml
ACE2-HEK293 Recombinant Cell Line	79951	2 vials

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