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Data Sheet

Bald Lentiviral Pseudovirion (Luc-eGFP Dual Reporter)

Catalog#: 79988

Product Description

The bald lentiviral pseudovirion was produced without envelope glycoproteins such as VSV-G or SARS-CoV-2 spike. It contains a firefly luciferase and eGFP cassette (Luc-P2A-eGFP) as the reporters, driven by a CMV promoter (Figure 1). The bald lentiviral pseudovirion can serve as a negative control when studying virus entry initiated by specific interactions between virus particles and receptors.

Application

Ideal as a negative control pseudovirion for the Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luc-eGFP Dual Reporter), BPS Bioscience #79982 or other pseudovirions used to study the mechanism of viral transduction.

Formulation

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

Titer

Since the virus is lacking the envelope glycoproteins and cannot transduce target cells, functional titer of this product cannot be determined. Based on p24 values, the approximate number of lentiviral particles (LP) of this product is ~ 10^9 LP/ml.

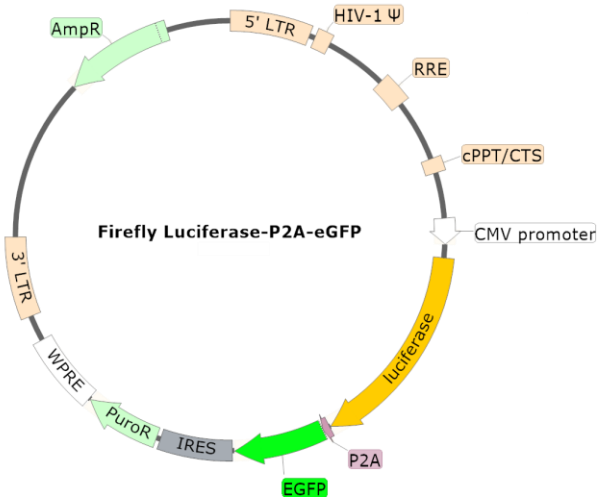

Figure 1. Schematic of the Luciferase-P2A-eGFP Reporter in Bald Lentiviral Pseudovirion

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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 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)
- SARS-CoV-2 Spike Pseudotyped Lentivirus (Luc-eGFP dual reporter) (BPS Bioscience, #79982)
- 96-well tissue culture treated, white clear-bottom assay plate (Corning, #3610)
- ONE-Step™ luciferase assay system (BPS Bioscience, #60690)

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) and the corresponding control bald lentiviral pseudovirion (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.

   For control cells to demonstrate the transduction is dependent upon ACE2, the same number of HEK293 parental cells were seeded.

2. Day 2: To each well add 5 µl of SARS-CoV-2 Spike Pseudotyped Lentivirus or 5 µl of Bald Lentiviral Pseudovirion. Optional: Add polybrene to each well at a final concentration of 5 µg/ml. Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2 for 18-24 hours.

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

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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, 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.

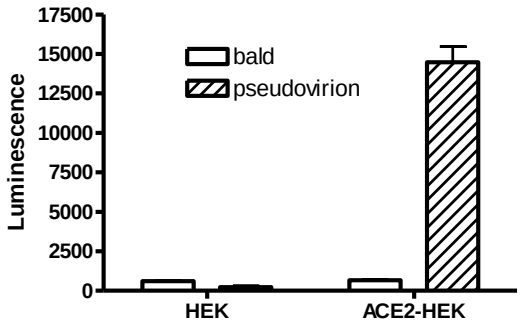

Figure 2. Transduction of ACE2-HEK293 Cells Monitored by Luciferase Activity.
Approximately 10,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luc-eGFP dual reporter) or bald lentiviral pseudovirion (Luc-eGFP dual 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.

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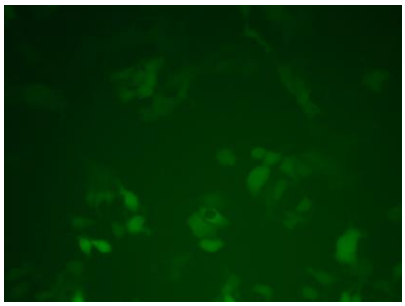

Figure 3. Transduction of ACE2-HEK293 Cells Monitored by eGFP Expression. Approximately 10,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 20 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luc-eGFP dual reporter) or bald lentiviral pseudovirion (Luc-eGFP dual reporter). 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, 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) (data not shown), indicating the transduction is dependent upon the ACE2 receptor.

Related Products

Product	Cat. #	Size
SARS-CoV-2 Spike Pseudotyped Lentivirus (Luciferase-eGFP dual Reporter)	79982	500 µl x2
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
eGFP Lentivirus	79979	500 µl x2
Firefly Luciferase-eGFP Lentivirus	79980	500 µl x2
NFκB Luciferase Reporter Lentivirus	79564	500 µl x2
IL-2 Promoter Luciferase Reporter Lentivirus	79825	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