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Data Sheet

Bald Lentiviral Pseudovirion (Luciferase Reporter)

Catalog#: 79943

Product Description
The bald lentiviral pseudovirion was produced without envelope glycoproteins such as VSV-G or SARS-CoV-2 spike. It contains the firefly luciferase gene driven by a CMV promoter (Figure 1) as the reporter. 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 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 lentiviral particles (LP) of this product is ~ 10^9 LP/ml.

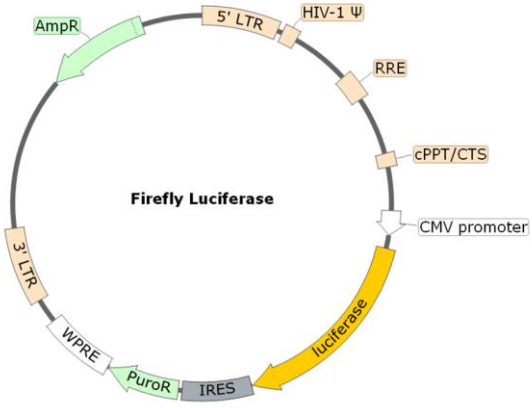

Figure 1. Schematic of the Luciferase Reporter in Bald Lentiviral Pseudovirion (Luciferase Reporter)

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.

Materials Required but Not Supplied

- HEK293 growth medium or use
  Thaw Medium 9 (BPS Bioscience #79665): MEM with 10% FBS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate
- HEK293 cells (ATCC#CRL-1573)
- ACE2-HEK293 Recombinant Cell Line (BPS Bioscience, #79951)
- SARS-CoV-2 Spike Pseudotyped lentivirus (Luciferase reporter) (BPS Bioscience, #79942)
- Polybrene (Millipore, # TR-1003-G)
- 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 HEK293 cells. 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 HEK293 cells or 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 9 (BPS Bioscience, #79665). Incubate cells at 37°C with 5% CO_2 overnight.

2. Day 2: To each well add 5 µl of SARS-CoV-2 Spike Pseudotyped Lentivirus or 5 µl of Bald Lentiviral Pseudovirion. 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% CO2 for 18-24 hours.

   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 9 to each well.

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 efficiency is determined by measuring the luciferase activity.

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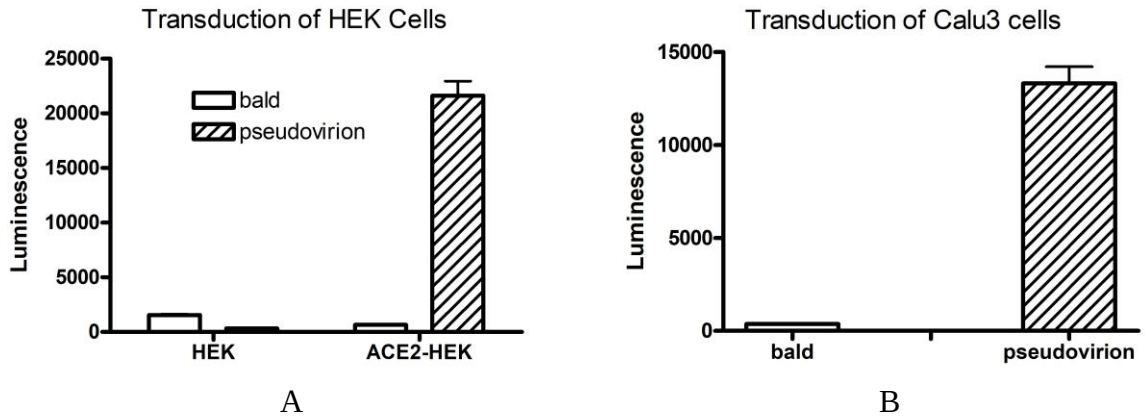

Figure 2. Transduction of ACE2-HEK293 cells and Calu3 cells using SARS-CoV-2 Spike pseudotyped lentivirus.

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 reporter) (BPS Bioscience #79942). After 18 hours of transduction, the medium was changed to fresh HEK growth medium (Thaw Medium 9). 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 with much greater efficiency compared with HEK293 parental cells, indicating the transduction is dependent upon ACE2 expression. The bald lentiviral pseudovirion, where no envelope glycoprotein is expressed, was used as a negative control.

B. Approximately 25,000 Calu3 cells/well were transduced with 5 µl/well of SARS-CoV-2 Spike pseudotyped lentivirus (Luc reporter) in the presence of 5 µg/ml of polybrene. After 18 hours of transduction, the medium was changed to fresh Calu3 growth medium. After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience, #60690) was added to cells to measure the luciferase activity. The bald lentiviral pseudovirion, where no envelope glycoprotein is expressed, was used as a negative control.

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Related Products

Product	Cat. #	Size
SARS-CoV-2 Spike Pseudotyped Lentivirus (Luciferase Reporter)	79942	500 µl x2
NFκB Luciferase Reporter Lentivirus	79564	500 µl x2
CRE Luciferase Reporter Lentivirus	79580	500 µl x2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x2
TCF/LEF Luciferase Reporter Lentivirus	79787	500 µl x2
ISRE Luciferase Reporter Lentivirus	79824	500 µl x2
IL-2 Promoter Luciferase Reporter Lentivirus	79825	500 µl x2
IL-8 Promoter Luciferase Reporter Lentivirus	79827	500 µl x2
AP-1 Luciferase Reporter Lentivirus	79823	500 µl x2
SBE Luciferase Reporter Lentivirus	79806	500 µl x2
TEAD Luciferase Reporter Lentivirus	79833	500 µl x2
ARE Luciferase Reporter Lentivirus	79869	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