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Data Sheet

Firefly Luciferase-eGFP Lentivirus (G418)

Catalog#: 79980-G

Product Description
The Firefly Luciferase-eGFP Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types mammalian cells, including primary and non-dividing cells. These viruses contain a firefly luciferase and eGFP cassette (Luc-P2A-eGFP) driven by a CMV promoter (Figure 1). Both the luciferase and eGFP are coexpressed under the CMV promoter in the transduced cells, allowing greater flexibility for detection of transduced cells.

Application
Ideal as a positive control for transduction; useful for transduction optimization.

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

Titer
Two vials (500 µl x 2) of Firefly Luciferase-eGFP lentivirus at a titer ≥1 x 10^7 TU/ml. 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.

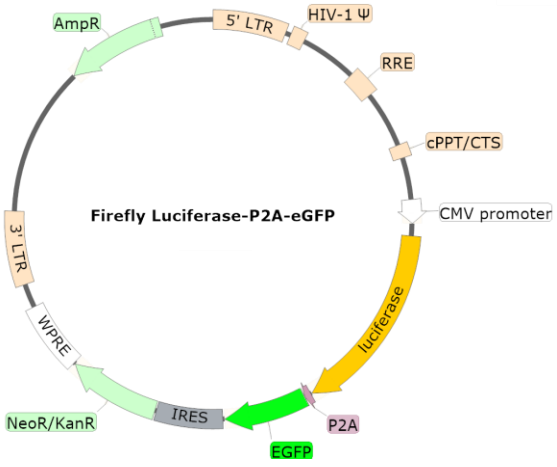

Figure 1. Schematic of lenti-vector used to generate the firefly luciferase-eGFP lentivirus

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Biosafety
The lentiviruses are produced with the third generation SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. 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).
- 96-well tissue culture treated, white clear-bottom assay plate (Corning, #3610)
- ONE-Step™ luciferase assay system (BPS Bioscience, #60690)
- Luminometer

Assay Protocol
The following protocol is a general guideline for transducing HEK293 cells using Firefly Luciferase-eGFP lentivirus. 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 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.

2. Day 2: To each well add 5 µl Firefly Luciferase-eGFP lentivirus. 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.

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 the medium.

4. Day 4, approximately 48-60 hours after transduction, prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 50 µl ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence

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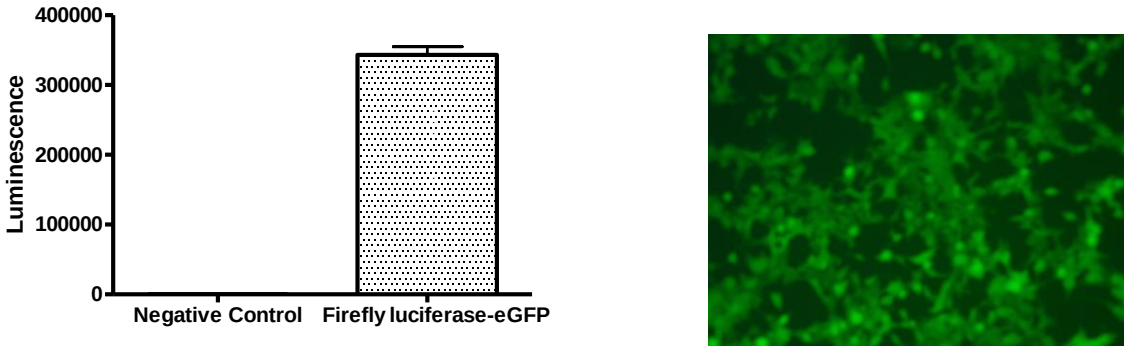
using a luminometer. The transduction efficacy is determined by measuring the luciferase activity.

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. eGFP has an excitation wavelength of 488 nm, an emission wavelength of 509 nm, and an extinction coefficient of 55,000 $M^{-1}cm^{-1}$.

Important Notes:

To generate the Firefly Luciferase-eGFP stable cell line, on day 4 remove HEK growth medium and replace it with fresh growth medium containing the appropriate amount of G418 for antibiotic selection of transduced cells.



A B

Figure 2. Transduction of HEK293 Cells Monitored by Luciferase Activity and eGFP expression.

A. Approximately 10,000 cells/well of HEK293 cells were transduced with 5 µl/well of Firefly Luciferase-eGFP lentivirus or expression negative control lentivirus (BPS Bioscience #79902-G). 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.

B. Approximately 10,000 cells/well of HEK293 cells were transduced with 5 µl/well of Firefly Luciferase-eGFP lentivirus. After 18 hours of transduction, the medium was changed to fresh HEK growth medium (Thaw Medium 1). After 48 hours of transduction, the expression of eGFP in the target cells was examined using fluorescence microscopy.

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Related Products

Product	Cat. #	Size
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
SARS-CoV-2 Spike Pseudotyped Lentivirus (Luciferase-eGFP Reporter)	79982	500 µl x2
Bald Lentiviral Pseudoviron (eGFP Reporter)	79987	500 µl x2
Bald Lentiviral Pseudoviron (Luciferase-eGFP dual Reporter)	79988	500 µl x2
eGFP Lentivirus	79979	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
Expression negative Control Lentivirus	79902	500 µl x2
NFAT eGFP Reporter Lentivirus	79922	500 µl x2