Description

The Firefly Luciferase-eGFP Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to infect almost all types of mammalian cells, including primary and non-dividing cells. These viruses transduce firefly luciferase and eGFP (Luc2-P2A-eGFP) driven by an EF1A promoter (Figure 1). The luciferase and eGFP proteins are co-expressed under the EF1A promoter in the cells, allowing greater flexibility for detection of the transduced cells.

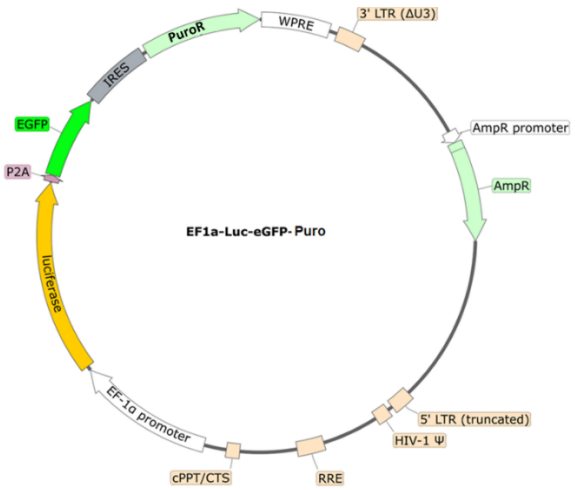

Figure 1. Schematic of the lenti-vector used to generate the Firefly Luciferase-eGFP Lentivirus.

Application

- Ideal as a positive control for transduction
- Optimize transduction assays and track reporter protein expression over time.
- Generate a stable cell line expressing Firefly Luciferase and eGFP with Puromycin selection.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 µl x 2) of lentivirus at a titer ≥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 lentiviruses at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with a SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after 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 and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Materials Required but Not Supplied

These materials are not supplied with this lentivirus but are necessary to follow the protocol described in the "Validation Data" section. Media and reagents used at BPS Bioscience are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Polybrene	Millipore #TR-1003-G
One-Step Luciferase Assay System	BPS Bioscience #60690
96-well clear-bottom tissue culture-treated assay plates (clear-bottom and white for luminescence assay)	
Flow cytometer or fluorescence microscope	
Luminometer	

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using the 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 target gene can be measured approximately 48-72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the target gene with the appropriate antibiotic prior to carrying out the reporter assays.

1. Day 1: Seed HEK293 cells at a density of 5,000-10,000 cells per well into a clear-bottom 96-well cell culture plate in 100 µl of Thaw Medium 1 (BPS Bioscience #60187). Incubate the cells at 37°C with 5% CO_2 overnight.

2. Day 2: Add 1 µl of Firefly Luciferase-eGFP lentivirus into each well. 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 overnight.

   Alternatively, cell seeding and transduction can be performed at the same time.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 100 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 can be examined under a fluorescence microscope or by flow cytometry.

   To measure the luciferase activity, add the ONE-Step™ Luciferase assay reagent (BPS Bioscience #60690), following the recommended protocol.

Notes

To generate a Firefly Luciferase-eGFP reporter stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of Puromycin (as pre-determined from a killing curve) for antibiotics selection of transduced cells. Visit Cell Line FAQs (bpsbioscience.com) "What is a kill curve?" for more information.

Figures and Validation Data

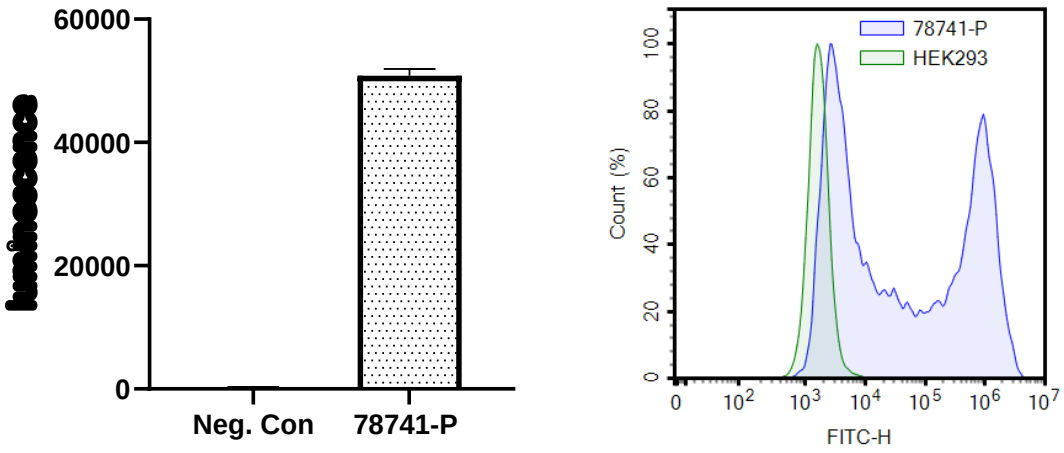

Figure 2: Luciferase activity and eGFP expression in transduced HEK293 cells.
Left: 10,000 HEK293 cells were infected with 0.5 µl of Firefly Luciferase-eGFP Lentivirus or Negative Control Lentivirus (BPS Bioscience #79902-P). After 66 hours of transduction, the ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure the luciferase activity. Right: 10,000 HEK293 cells were infected with 0.5 µl of Firefly Luciferase-eGFP lentivirus. After 66 hours of transduction, the expression of eGFP was analyzed by Flow Cytometry.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Firefly Luciferase-eGFP Lentivirus	79980	500 µl x 2
Enhanced GFP Lentivirus (G-H)	78639	500 µl x 2
Firefly Luciferase Lentivirus	79692	500 µl x 2
Renilla Luciferase Lentivirus	79565	500 µl x 2