

Description

Red Shifted Firefly Luciferase Lentivirus are replication incompetent, HIV based, VSV-G pseudotyped lentiviral particles that are ready to transduce almost all types of mammalian cells, including primary and non-dividing cells. These particles contain a red shifted firefly luciferase (*Luciola italica*) under the control of an EF1a promoter. The lentiviruses also transduce a puromycin selection marker (Figure 1). Luciferase expression and transduction efficiency can easily be verified and optimized by using a luminometer.

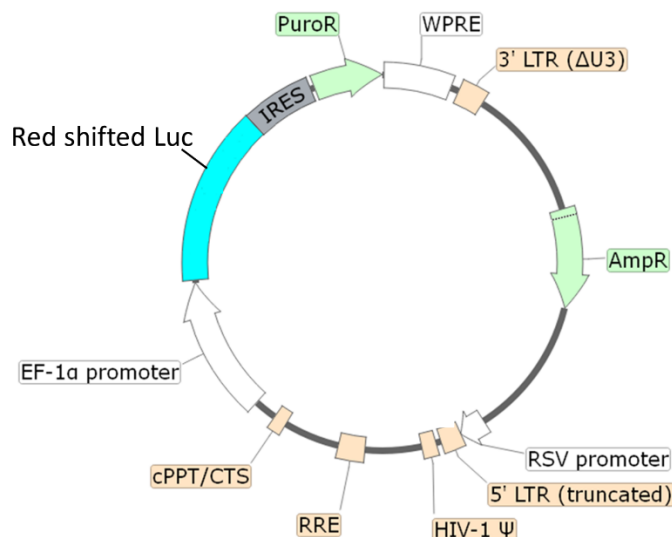


Figure 1. Schematic of the lenti-vector used to generate the Red Shifted Firefly Luciferase Lentivirus.

Background

Luciferase is an enzyme that catalyzes a multi-step chemical reaction using luciferin to generate light. The ability to generate bioluminescence has made luciferase a crucial biomedical reagent, allowing easy read outs in cellular and biochemical assays. One of the most used is *Photinus pyralis* luciferase, which is stable and has a rapid turnover. Its emission peak falls in the yellow-green range, which coincides with the absorption spectra of molecules like hemoglobin and melanin. The use and/or development of a red-shifted luciferase can improve deep-tissue penetration and sensitivity. Italic firely *Luciola italica* has a red-shifted luciferase with an increase in quantum yield. These properties make a suitable reagent for *in vivo* deep imaging.

Application(s)

- Generation of cell pools or stable cell lines expressing red shifted luciferase following puromycin selection.
- *In vitro* and *in vivo* Bioluminescence Imaging.

Formulation

The lentivirus particles were produced in HEK293T cells in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Size and Titer

Two vials (500 μ l x 2) of lentivirus at a titer $\geq 10^7$ 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 for up to 12 months from date of receipt. 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
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well clear-bottom tissue culture-treated assay plates	
Flow cytometer or fluorescence microscope	

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Media Required for the Proposed Assay

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using Red Shifted Luciferase 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 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 with the appropriate antibiotic prior to carrying out the reporter assays.

Day 1:

1. Harvest HEK293 cells from culture and seed cells at a density of 5,000-10,000 cells per well in 100 μl of Thaw Medium 1 into a clear-bottom 96-well microplate.

2. Add 1 μ l of Red Shifted Firefly Luciferase lentiviruses into each well.
3. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each well to a final concentration of 5 μ g/ml.
4. Gently swirl the plate to mix.
5. Incubate the plate at 37°C with 5% CO₂ overnight.

Day 2:

1. Remove the medium containing the lentivirus from the wells.
2. Add 100 μ l of fresh Thaw Medium 1 to each well.

Note: If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 2. The target cells can be incubated with the virus for 48-72 hours before changing medium.

Day 3-4:

1. 48-72 hours post-transduction, the transduced cells are ready for analysis by the methods of interest.
2. To measure the luciferase activity, add the ONE-Step™ Luciferase assay reagent (BPS Bioscience #60690), following the recommended protocol.

Notes

To generate a red shifted luciferase expressing 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, [Kill Curve Protocol](#)), for antibiotic selection of transduced cells, followed by clonal selection.

Figures and Validation Data

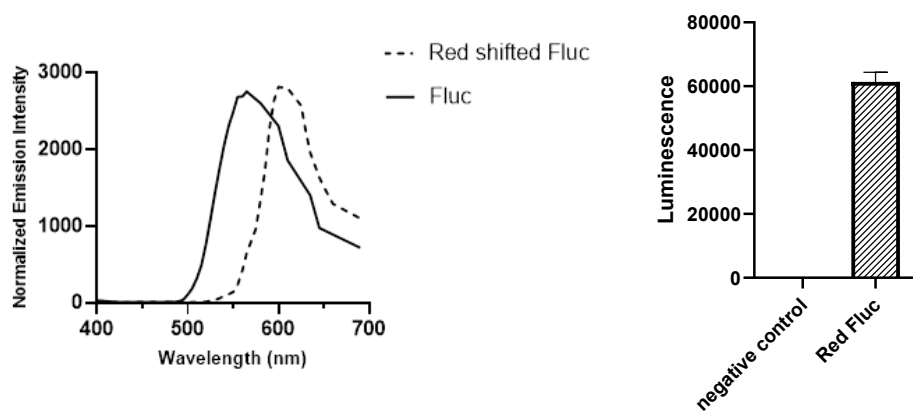


Figure 2. Expression of red shifted luciferase in HEK293 cells transduced with Red Shifted Firefly Luciferase Lentivirus.

Approximately 10,000 cells/well of HEK293 cells were transduced with 1 μ l/well of Red Shifted Firefly Luciferase Lentivirus. 66 hours post-transduction, ONE-Step™ Luciferase Assay reagent was added for ~15 to 30 minutes.

Left: The bioluminescence emission spectrum from red shifted firefly luciferase was scanned using a luminometer and compared with firefly luciferase from *Photinus pyralis* (Fluc). Fluc has an emission maximum of ~560 nm while red shifted firefly luciferase from *Luciola italica* has an emission maximum of ~610 nm.

Right: Luminescence of transduced and non-transduced cells was measured using a luminometer.

Data shown is representative.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

Related Products

Products	Catalog #	Size
Firefly Luciferase-eGFP Lentivirus (G418 or Puromycin)	79980	500 μ l x 2
Enhanced GFP Lentivirus (G418, Hygromycin and Puromycin)	78639	500 μ l x 2
Firefly Luciferase (G418, Hygromycin and Puromycin)	79692	500 μ l x 2
Renilla Luciferase Lentivirus (G418 or Puromycin)	79565	500 μ l x 2
Expression Negative Control Lentivirus (G418 or Hygromycin or Puromycin)	79902	500 μ l x 2
Firefly Luciferase-eGFP Lentivirus (EF1A Promoter/Geneticin, Hygromycin or Puromycin)	78740	500 μ l x 2

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