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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Firefly Luciferase-Nuclear eGFP 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 firefly luciferase and nuclear eGFP (Luc2-P2A-nuclear eGFP) driven by an EF1A promoter. These lentiviruses also transduce a puromycin selection marker (Figure 1). The nuclear eGFP has NLS sequences at the C-terminus. eGFP has an excitation wavelength of 488 nm, an emission wavelength of 509 nm, and extinction coefficient of 55,000 $M^{-1}cm^{-1}$.

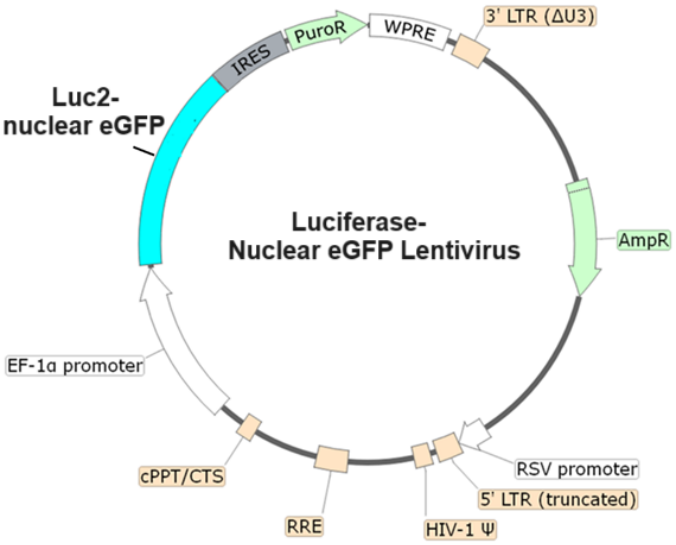

Figure 1. Schematic of the lenti-vector used to generate the Firefly Luciferase-Nuclear eGFP Lentivirus.

Background

GFP (green fluorescent protein) presents green fluorescence, and it was first identified in *Aequorea Victoria*. It has become widely used in cell biology to monitor gene expression, protein localization, and protein-protein interactions. Its popularity prompted the development of variants, such as the eGFP (enhanced GFP). eGFP has a higher intensity emission versus the GFP molecule. Nuclear localization signal (NLS) sequences have been used for artificial localization of green fluorescent protein (GFP) in the nucleus. The presence of firefly luciferase allows for easy assay read-outs using luminescence.

Application

- Sub-cellular (Nuclear) labelling of cells
- Generation of cell pools or stable cell lines expressing firefly luciferase and nuclear eGFP following puromycin selection.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS . Virus particles can be packaged in custom formulations and produced at higher titers by special request, for an additional fee.

Size and 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 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
96-well tissue culture-treated assay plates	
One-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	
Flow cytometer or fluorescence microscope	

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using the Firefly Luciferase-Nuclear 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 puromycin 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 white opaque 96-well microplate.

2. Add 5 µl of Firefly Luciferase-Nuclear eGFP Lentivirus into each well.

3. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each well to a final concentration of 5 µg/ml.

4. Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2.

Day 3 or 4:

1. The expression of eGFP can be analyzed by microscopy or flow cytometry (Ex/Em=488/510 nm), or another method of interest.

2. Prepare the ONE-Step™ Luciferase reagent as recommended in the product protocol (#60690) (100 µl/well). Add 100 µl of ONE-Step™ Luciferase Assay reagent per well.

3. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Notes

To generate a Firefly Luciferase-Nuclear eGFP 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, https://bpsbioscience.com/kill-curve-protocol), for antibiotic selection of transduced cells, followed by clonal selection.

Figures and Validation Data

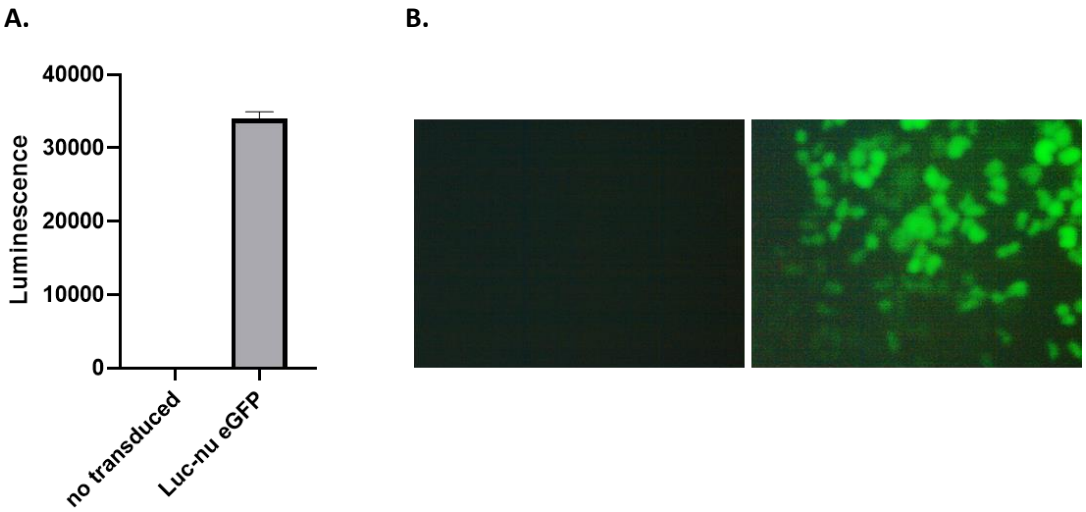

Figure 2. Firefly luciferase and nuclear eGFP expression in HEK293 cells transduced with Firefly Luciferase-Nuclear eGFP lentivirus.
Approximately 5,000 HEK293 cells/well were transduced with 10 µl/well of Firefly Luciferase-Nuclear eGFP Lentivirus. 66 hours post-transduction, **(A)** the luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690); **(B)** the expression of eGFP in the nucleus of the target cells was observed under a fluorescence microscope (left panel: un-transduced HEK293 cells; right panel: HEK293 cells transduced with Firefly Luciferase-Nuclear eGFP lentivirus

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 (G418) or (Puromycin)	79980	500 µl x 2
Firefly Luciferase Lentivirus (G418, Hygromycin and Puromycin)	79692	500 µl x 2
Enhanced GFP Lentivirus (G418, Hygromycin and Puromycin)	78639	500 µl x 2
Nuclear eGFP Lentivirus (Puromycin)	78976	500 µl x 2

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