Description

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 a nuclear eGFP (enhanced green fluorescent protein), with nuclear localization sequences (NLS) at both N and C-terminus of eGFP), under the control of an EF1a promoter. The lentiviruses also transduce a puromycin selection marker (Figure 1). eGFP expression and transduction efficiency can easily be verified and optimized via fluorescence microscopy or flow cytometry. eGFP has an excitation wavelength of 488 nm, an emission wavelength of 509 nm, with an extinction coefficient of 55,000 $M^{-1}cm^{-1}$.

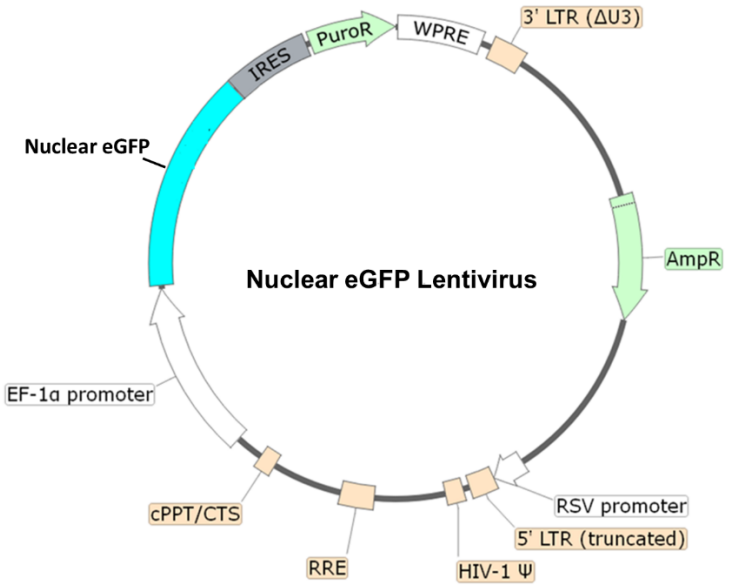

Figure 1. Schematic of the lenti-vector used to generate the Nuclear eGFP Lentivirus.

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 mutant 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.

Application

- Nuclear labelling.
- Generation of cell pools or stable cell lines expressing nuclear eGFP following puromycin selection.

Formulation

The lentiviruses were produced from HEK293T cells. Supplied in 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
HEK293 Cells	ATCC #CRL-1573
Thaw Medium 1	BPS Bioscience #60187
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
96-well tissue culture-treated assay plates	
Flow cytometer or fluorescence microscope	

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 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 with puromycin prior to carrying out the assays.

Day 1:

1. Harvest HEK293 cells from culture, centrifuge, and resuspend the cells in fresh Thaw Medium 1.
2. Count cells.
3. Plate HEK293 cells at a density of 5,000-10,000 cells per well into a 96-well cell culture plate in 100 µl of Thaw Medium 1.
4. Incubate the plate at 37°C with 5% CO_2 overnight.

Day 2:

1. Add 5 µl of Nuclear eGFP Lentivirus to the cells.

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

3. Gently swirl the plate to mix.

4. Incubate the plate at 37°C with 5% CO_2 overnight.

   Note: Alternatively, seeding cells and transduction can be performed on the same day.

Day 3:

1. Remove medium and add 100 µl of fresh Thaw Medium 1.

   Note: If neither the polybrene nor the lentivirus adversely affect 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.

2. Incubate the plate at 37°C with 5% CO_2 for 24-48 hours.

Day 4-5:

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

Notes

To generate a Nuclear eGFP 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, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, followed by clonal selection.

Figures and Validation Data

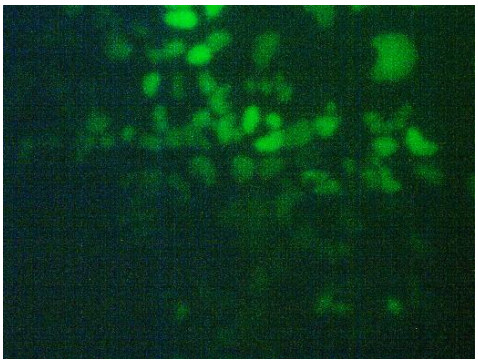

Figure 2. eGFP expression in HEK293 cells transduced with Nuclear eGFP Lentivirus.
Approximately 5,000 HEK293 cells/well were transduced with 5 µl/well of Nuclear eGFP Lentivirus. 66 hours post-transduction, the expression of eGFP in the nucleus of the target cells was observed under a fluorescence microscope.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

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*
eGFP Lentivirus (Inducible TET On)	78629	500 µl x 2
Enhanced GFP Lentivirus (G418)	78639-G	500 µl x 2
Enhanced GFP Lentivirus (Puro)	78639-P	500 µl x 2
Firefly Luciferase Lentivirus	79692	500 µl x 2
Renilla Luciferase Lentivirus	79565	500 µl x 2
Secreted Gaussia Lentivirus	79892	500 µl x 2

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