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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Mitochondrial 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 eGFP (enhanced green fluorescent protein), with a mitochondrial targeting sequence at the N-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.

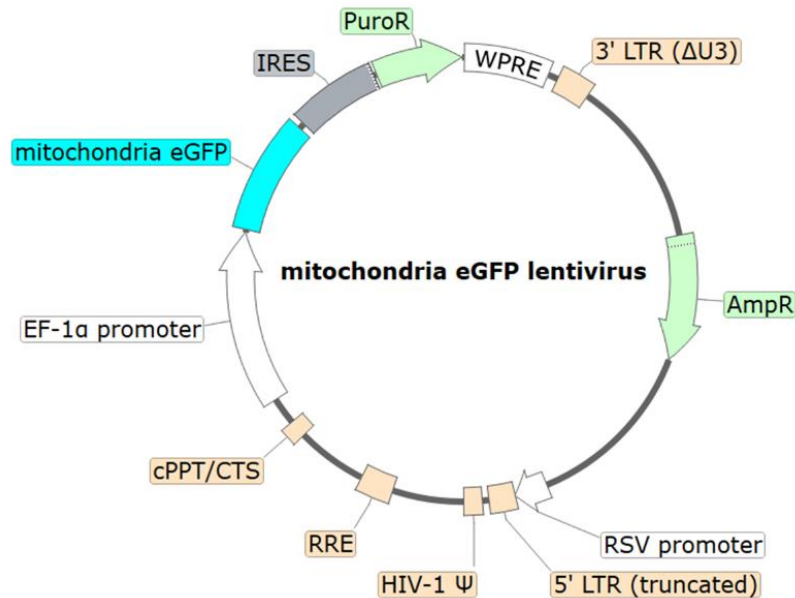


Figure 1. Schematic of the lenti-vector used to generate the Mitochondria eGFP Lentivirus.

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. A mitochondrial targeting sequence has been used for artificial localization of green fluorescent protein (GFP) to the mitochondria.

Application

- Mitochondrial labelling.
- Generation of cell pools or stable cell lines expressing mitochondrial 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 by special request, for an additional fee.

Titer

Two vials (50 µl x 2) of lentivirus at a titer $\geq 1 \times 10^8$ 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 the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Name	Ordering Information
HeLa Cells	ATCC # CCL-2
Thaw Medium 1	BPS Bioscience #60187
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
Glass Coverslips, Round	Fisher Scientific #08-774-387
Paraformaldehyde, 4% in PBS	Fisher Scientific #AAJ61899AK
Antifade Mounting Medium with DAPI	Vector Laboratories cat#H150010
Flow cytometer or fluorescence microscope	

Assay Protocol

The following protocol was used to transduce HeLa 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. Add coverslips to a 6-well plate using sterilized tweezers, if needed.
2. Harvest HeLa cells from culture, centrifuge, and resuspend the cells in fresh Thaw Medium 1.
3. Count cells and plate at a density of 100,000 cells per well into a 6-well cell culture plate in 2ml of Thaw Medium 1.

Day 2:

1. Add 5 μ l of Mitochondrial eGFP Lentivirus to the cells.
2. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each well to a final concentration of 5 μ g/ml.
3. Gently swirl the plate to mix, incubate the plate at 37°C with 5% CO₂ overnight.

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

Day 3:

1. Remove medium and add 2 ml 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% CO₂ for 24-48 hours.

Day 4-5:

1. Remove the medium and wash the cells with PBS.
2. Add 4% PFA (paraformaldehyde) to each well and incubate for 15 minutes.
3. Remove the fixative and wash twice with PBS.
4. Mount the coverslips onto microscope slides with mounting medium with DAPI.
5. The expression of mitochondrial eGFP can be monitored by microscopy.

Note: Fluorescence can also be analyzed by flow cytometry, or another method of interest.

Figures and Validation Data

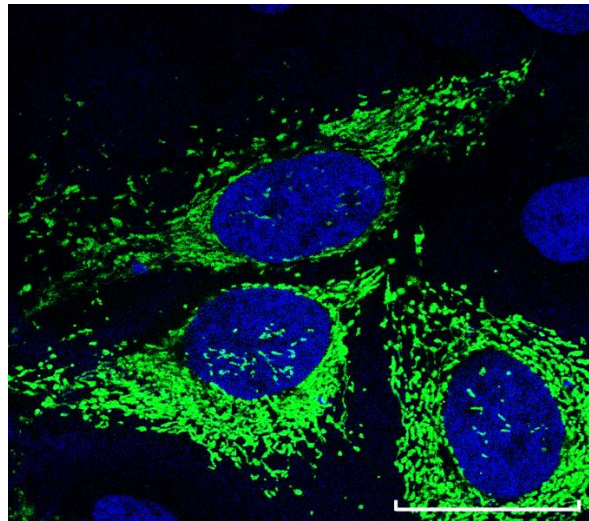


Figure 2. eGFP expression in HeLa cells transduced with Mitochondrial eGFP Lentivirus. Approximately 100,000 HeLa cells were plated on coverslips and transduced with Mitochondrial eGFP Lentivirus at a MOI of 10. 48 hours post-transduction, the cells were fixed with 4% PFA, the cover slips with cells were mounted onto microscope slides by using mounting medium with DAPI (Vector Laboratories cat#H150010). The image was captured using a confocal microscope. Green: Mitochondrial eGFP; Blue: Nucleus; Scale bar: 50 μ m.

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

Troubleshooting Guide

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

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Nuclear eGFP Lentivirus (Puromycin)	78976	50 μ l x 2
Endoplasmic Reticulum eGFP Lentivirus	82326	50 μ l x 2
Membrane eGFP Lentivirus	82324	50 μ l x 2
eGFP Beta-Actin Lentivirus	82327	50 μ l x 2
eGFP-Tubulin Lentivirus	82328	50 μ l x 2
Nuclear mCherry Lentivirus	82334	50 μ l x 2

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