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Produktinformation

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

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Nuclear mCherry 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 mCherry, with nuclear localization sequences (NLS) at both the N- and C-terminus of mCherry, under the control of an EF1a promoter. The lentiviruses also transduce a puromycin selection marker (Figure 1). mCherry expression and transduction efficiency can easily be verified and optimized via fluorescence microscopy or flow cytometry.

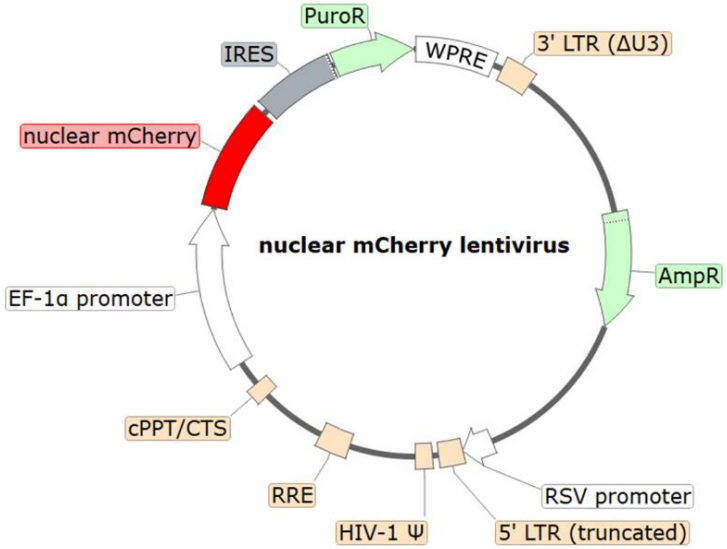

Figure 1. Schematic of the lenti-vector used to generate the Nuclear mCherry Lentivirus.

Background

mCherry is a monomeric red fluorescent protein derived from DsRed found in the sea anemones Discosoma. It belongs to the mFruit family of monomeric red fluorescent proteins, which are improved versions of mRFP1 (monomeric red fluorescent protein 1) in terms of brightness and photostability. The use of fluorescent proteins allows for direct visualization of transfected or transduced cells under a fluorescent microscope or analysis by flow cytometry. The use of lentiviruses to introduce mCherry is a convenient strategy that allows expression of the markers in almost all mammalian cells and to easily determine transduction efficiency. Nuclear localization signal (NLS) sequences have been used for artificial localization of red fluorescent protein (mCherry) to the nucleus.

Application

- Nuclear labelling.
- Generation of cell pools or stable cell lines expressing Nuclear mCherry 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 ≥1 x 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 #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 2 ml of Thaw Medium 1.

Day 2:

1. Add 5 µl of Nuclear mCherry 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, 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 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% CO_2 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 nuclear mCherry can be monitored by microscopy.

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

Figures and Validation Data

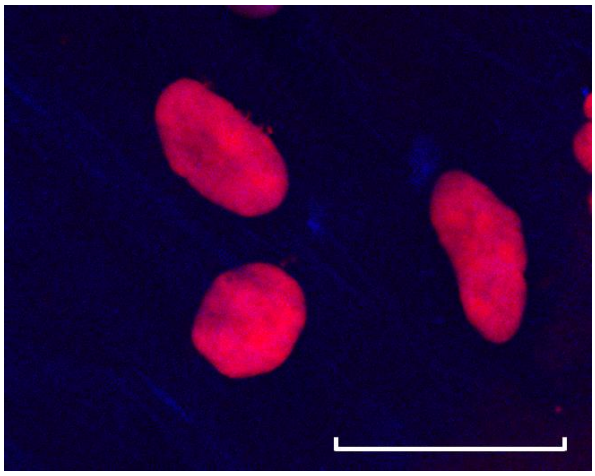

Figure 2. mCherry expression in HeLa cells transduced with Nuclear mCherry Lentivirus. Approximately 100,000 HeLa cells were plated on coverslips and transduced with Nuclear mCherry Lentivirus at a MOI of 10. 48 hours post-transduction, the cells were fixed with 4% PFA, the cover slips with cells were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

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

Products	*Catalog #*	*Size*
Nuclear eGFP Lentivirus (Puromycin)	78976	50 µl x 2
Membrane eGFP Lentivirus	82324	50 µl x 2
Mitochondrial eGFP Lentivirus	82325	50 µl x 2
Endoplasmic Reticulum (ER) eGFP Lentivirus	82326	50 µl x 2
eGFP Beta-Actin Lentivirus	82327	50 µl x 2
eGFP-Tubulin Lentivirus	82328	50 µl x 2

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