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Produktinformation

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- 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

Enhanced green fluorescent protein (eGFP) is a modified (F64L and S65T mutations) version of the native GFP protein isolated from jellyfish (*Aequorea victoria*), displaying increased fluorescence and more efficient folding. Enhanced GFP Lentiviruses (Inducible TET On) are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to infect almost all types of mammalian cells, including primary and non-dividing cells. These viruses constitutively express eGFP under a tight TRE tetracycline-inducible promoter (Figure 1). After induction with Doxycycline, eGFP expression can easily be visualized and optimized via fluorescence microscopy or flow cytometry. eGFP has an excitation wavelength of 488 nm, an emission wavelength of 509 nm, and extinction coefficient of 55,000 $M^{-1}cm^{-1}$.

Application

1. Ideal as a positive control for transduction with a tetracycline-inducible expression system; useful for transduction optimization.
2. Generation of a TET inducible stable cell line expressing eGFP with G418 selection.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

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.

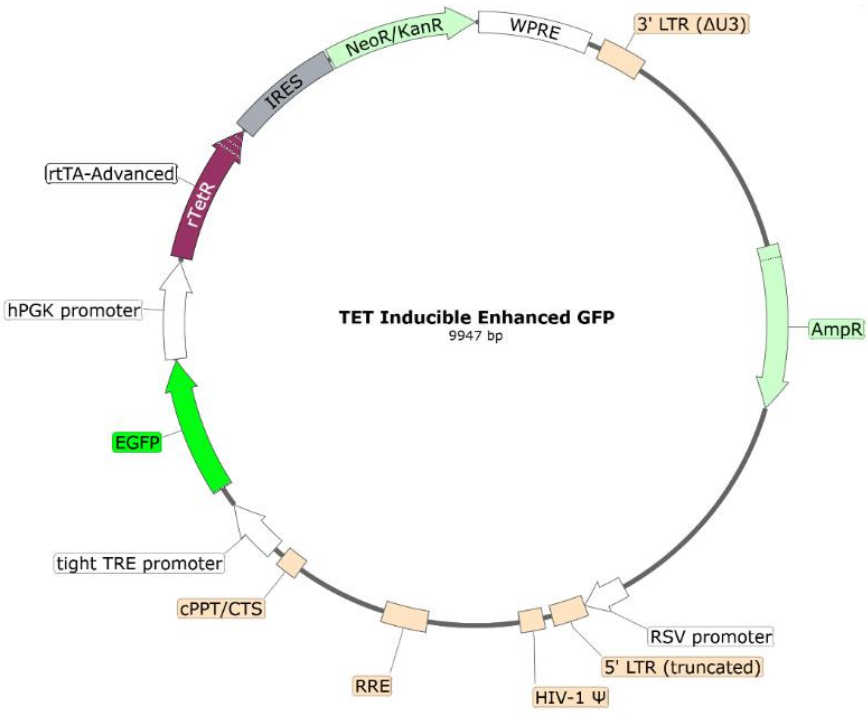

Figure 1. Schematic of the lenti-vector used for the eGFP lentivirus (Inducible TET on). Note that NeoR confers resistance to Geneticin (G418).

Storage

Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the lentiviruses at -80°C. 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. Thaw Medium 16 is manufactured with TET-free FBS to avoid promoter leakage.

Name	Ordering Information
HEK293 cells	ATCC #CRL-1573
Thaw Medium 16	BPS Bioscience #78647
Doxycycline	Sigma #D9891
Polybrene	Millipore, #TR-1003-G
96-well tissue culture-treated assay plates	
Flow cytometer or fluorescence microscope	

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance. To ensure the maximal range of induction, we recommend using serum which does not contain trace levels of tetracycline (or its derivatives).

Media Required for the Proposed Assay

Thaw Medium 16 (BPS Bioscience, #78647):

MEM medium supplemented with 10% TET-free 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 the eGFP lentivirus (Inducible TET on). 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 reporter 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 reporter gene with the appropriate antibiotic prior to carrying out the reporter assays.

1. Day 1: Harvest HEK293 cells from culture and seed cells at a density of 10,000 cells per well into a clear-bottom 96-well microplate in 100 µl of Thaw Medium 16 (BPS Bioscience #78647).

   a. Add 5 µl of TET Inducible eGFP lentivirus into each well. Gently swirl the plate to mix.

      Optional: Add polybrene to each well at a final concentration of 5 µg/ml.

   b. Incubate the plate at 37°C with 5% CO_2 for 24 hours.

2. Day 2: Remove the medium containing the lentivirus from the wells. Add 100 µl of fresh Thaw Medium 16 with varying concentrations of Doxycycline to each well.

3. Day 3-4, approximately 48-72 hours after transduction, the expression of enhanced GFP in the target cells can be examined under a fluorescence microscope or flow cytometry.

Notes

To generate the TET Inducible Enhanced GFP stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of G418 (as pre-determined from a killing curve) for antibiotic selection of transduced cells.

Figures and Validation Data

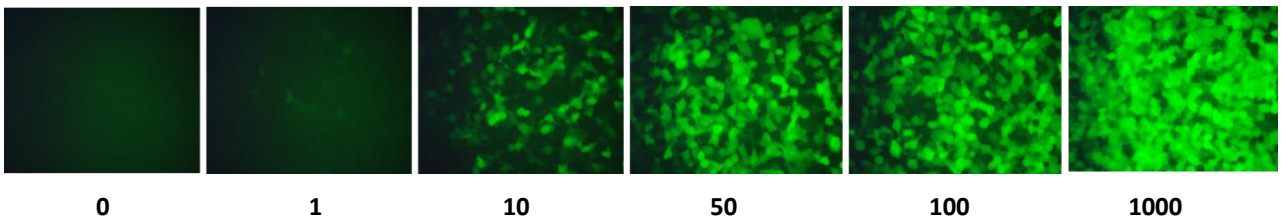

Figure 2. Transduction of HEK293 cells using eGFP lentivirus (Inducible TET On). Approximately 10,000 cells/well of HEK293 cells were transduced with 2 µl/well of Enhanced GFP lentivirus (Inducible TET on). After 24 hours of transduction, cells were treated with various concentrations of Doxycycline. After 24 hours of Doxycycline treatment, the expression of enhanced GFP in the target cells was observed under a fluorescence microscope.

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 Lentivirus	79692	500 µl x 2
Renilla Luciferase Lentivirus	79565	500 µl x 2
Secreted Gaussia Lentivirus	79892	500 µl x 2
Non-Secreted Gaussia Lentivirus	79893	500 µl x 2
Enhanced GFP Lentivirus	78639	500 µl x 2
YFP Lentivirus	79989	500 µl x 2
RFP Lentivirus	78347	500 µl x 2