

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 $55,000 \text{ M}^{-1}\text{cm}^{-1}$.

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 ($500 \mu\text{l} \times 2$) of lentivirus at a titer $\geq 10^7$ TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

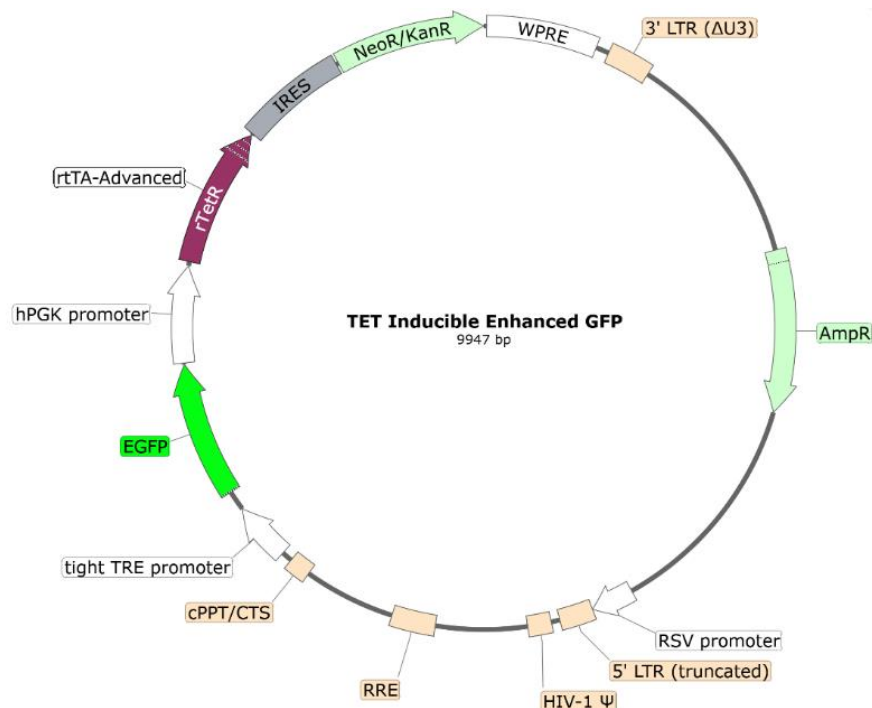


Figure 1. Schematic of the lenti-vector used for the eGFP lentivirus (Inducible TET on). Note that NeoR confers resistance to Geneticin (G418).

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 μ l of Thaw Medium 16 (BPS Bioscience #78647).
 - a. Add 5 μ l 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 μ g/ml.
 - b. Incubate the plate at 37°C with 5% CO₂ for 24 hours.
2. Day 2: Remove the medium containing the lentivirus from the wells. Add 100 μ l 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

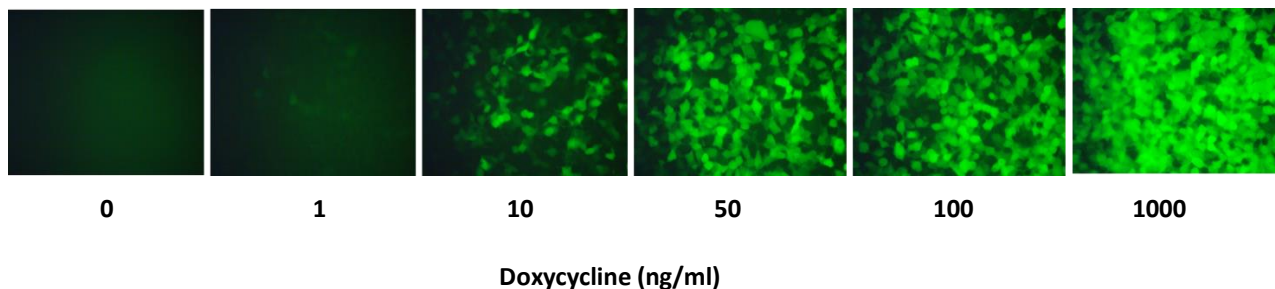


Figure 2. Transduction of HEK293 cells using eGFP lentivirus (Inducible TET On). Approximately 10,000 cells/well of HEK293 cells were transduced with 2 μ l/well of Enhanced GFP lentivirus (Inducible TET on). After 24 hours of transduction, cells were treated with various concentrations of Doxycycline. 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

| <i>Products</i> | <i>Catalog #</i> | <i>Size</i> |
|---------------------------------|------------------|-------------|
| 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 |