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Description

TurboRFP is a red (orange) fluorescent protein derived from sea anemone *Entacmaea quadricolor*. The RFP Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to infect almost all types mammalian cells, including primary and non-dividing cells. These viruses constitutively express TurboRFP under a CMV promoter (Figure 1). RFP expression and transduction efficiency can easily be verified and optimized via fluorescence microscopy or flow cytometry. RFP has an excitation wavelength of 553 nm, an emission wavelength of 574 nm, and an extinction coefficient of 92,000 $M^{-1}cm^{-1}$.

Application

- Ideal as a positive control for transduction; useful for transduction optimization.
- Generation of stable cells expressing RFP upon puromycin selection.

Formulation

The lentiviruses were produced from HEK293T cells. Supplied in medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 µl x 2) of RFP lentivirus at a titer ≥ 1 x 10^7 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

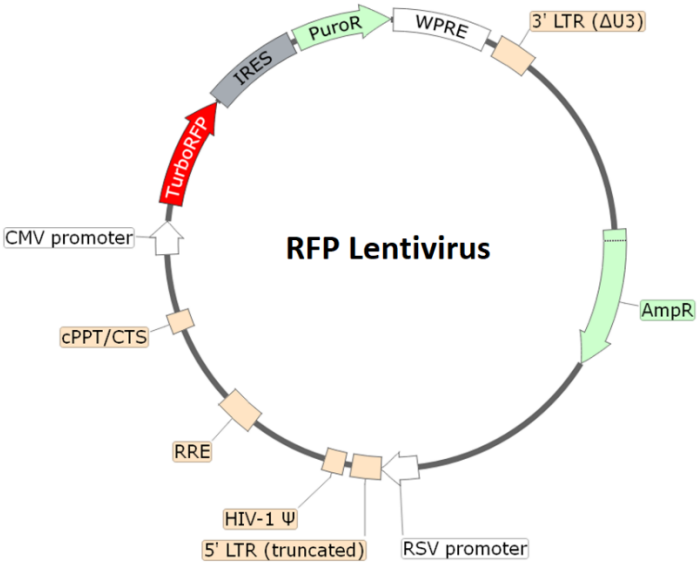

Figure 1. Schematic of the lenti-vector used to generate the RFP lentivirus

Storage

Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus 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 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. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS 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 designed protocol. BPS Bioscience media, reagents, and luciferase assay systems are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Polybrene	Millipore, # TR-1003-G
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 using RFP lentivirus. 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 gene with puromycin prior to performing 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 1 (BPS Bioscience, #60187). Incubate the cells at 37°C with 5% CO_2 overnight.

2. Day 2: Add 2 µl of RFP lentivirus into each well. Add polybrene to each well at a final concentration of 5 µg/ml. Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2 for 18-24 hours.

   Alternatively, cell seeding and transduction can be performed at the same day.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 100 µl of fresh Thaw Medium 1 to each well.

   If neither the polybrene nor the lentivirus adversely affects 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 medium.

4. Day 4-5, approximately 48-72 hours after transduction, the expression of RFP in the target cells can be examined under a fluorescence microscope or flow cytometry.

Important Notes:

To generate an RFP reporter 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 kill curve) for antibiotic selection of transduced cells.

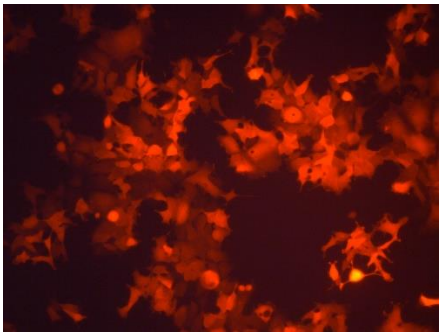

Figure 2. Transduction of HEK293 cells using RFP lentivirus. Approximately 10,000 cells/well of HEK293 cells were transduced with 2 µl/well of RFP lentivirus. After 66 hours of transduction, the expression of RFP in the target cells was observed under a fluorescence microscope.

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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 Luciferase Lentivirus	79893	500 µl x 2
Enhanced GFP Lentivirus	79979	500 µl x 2
YFP Lentivirus	79989	500 µl x 2