6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

Enhanced GFP Lentivirus (Puromycin)

Catalog #: 79979-P

Product Description

Enhanced green fluorescent protein (eGFP) is a modified (F64L and S65T mutations) version of the native GFP protein isolated from jellyfish (*Aequorea victoria*), with increased fluorescence and more efficient folding. The Enhanced GFP Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types mammalian cells, including primary and non-dividing cells. These viruses constitutively express eGFP under a CMV promoter (Figure 1). eGFP expression and transduction efficiency can easily be verified 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; useful for optimizing transduction assays and to track transduction efficiency over time.
2. Generation of stable cell line expressing eGFP with puromycin selection.

Formulation

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

Titer

Two vials (2 x 500 µl) of eGFP 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.

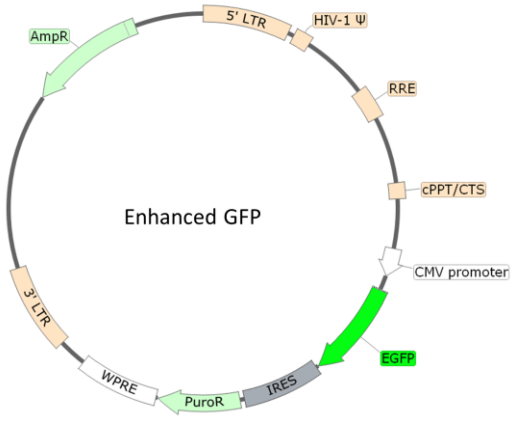

Figure 1. Schematic of the lenti-vector used to generate the eGFP lentivirus

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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
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
- HEK293 growth medium or use
  Thaw Medium 1 (BPS Bioscience #60187): MEM supplemented with 10% FBS, 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).
- Polybrene (Millipore, #TR-1003-G)
- 6-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 eGFP 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 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 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 white opaque 96-well microplate in 50 µl of Thaw Medium 1 (BPS Bioscience, #60187). Incubate cells at 37°C with 5% CO_2 overnight.

2. Day 2: Add 5 µl of eGFP 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, seeding cells and the transduction can be performed at the same day.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 50 µl of fresh Thaw Medium 1 to each well.

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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 eGFP in the target cells can be examined under a fluorescence microscope or flow cytometry.

   Important Notes:

   To generate the eGFP 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 for antibiotic selection of transduced cells.

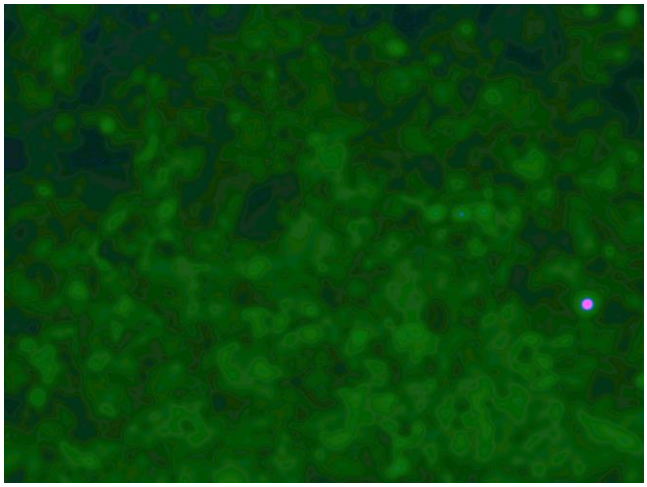

Figure 2. Transduction of HEK293 cells using eGFP lentivirus. Approximately 10,000 cells/well of HEK293 cells were transduced with 5 µl/well of eGFP lentivirus. After 18 hours of transduction, the medium was changed to fresh HEK293 growth medium (Thaw Medium 1). After 66 hours of transduction, the expression of eGFP in the target cells was observed under a fluorescence microscope.

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Related Products

Product	Cat. #	Size
YFP (Topaz) Reporter Lentivirus	79999	500 µl x2
NFAT/eGFP Reporter Lentivirus	79922	500 µl x2
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x2
SARS-CoV-2 Spike Pseudotyped Lentivirus (Luciferase Reporter)	79942	500 µl x2
Bald Lentiviral Pseudoviron (eGFP Reporter)	79987	500 µl x2
SARS-CoV-2 Spike Pseudotyped Lentivirus (eGFP Reporter)	79981	500 µl x2
Bald Lentiviral Pseudoviron (Luciferase-eGFP dual Reporter)	79988	500 µl x2
SARS-CoV-2 Spike Pseudotyped Lentivirus (Luciferase-eGFP Dual Reporter)	79982	500 µl x2
NFκB Luciferase Reporter Lentivirus	79564	500 µl x2
CRE Luciferase Reporter Lentivirus	79580	500 µl x2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (G418)	79692-G	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Hygromycin)	79692-H	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Puromycin)	79692-P	500 µl x2
FcGRIIIA Lentivirus	79876	500 µl x2
FcGRIIB Lentivirus	79877	500 µl x2
FcER1G Lentivirus	79878	500 µl x2
Expression negative Control Lentivirus	79902	500 µl x2
Secreted Gaussia Lentivirus	79892	500 µl x2
Non-Secreted Gaussia Luciferase Lentivirus	79893	500 µl x2
TCR Activator Lentivirus	79894	500 µl x2