Description

Expression Negative Control Lentivirus (EF1A Promoter/Puromycin) are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles ready to transduce nearly all types of mammalian cells, including primary and non-dividing cells. These viral particles do not express a specific protein but include only a puromycin selection marker under the control of the EF1A promoter (Figure 1).

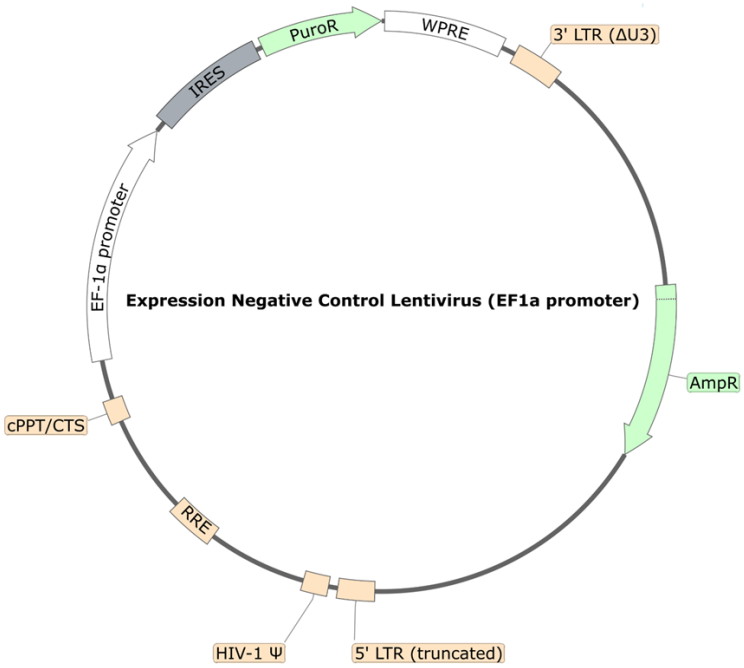

Figure 1. Schematic of the lenti-vector used to generate the Expression Negative Control Lentivirus (EF1A Promoter/Puromycin). This is a SIN vector.

Background

The use of antibiotics in cell culture as a method to establish stable cell lines by imposing selective pressure on cells expressing the antibiotic resistance is a common practice. However, the use of such antibiotics can result in altered gene expression and regulation. For instance, analysis of the impact of the use of penicillin-streptomycin identified 209 penicillin-streptomycin specific responsive genes, including transcription factors. In view of these findings, care should be taken when performing genetic, genomic and biological studies in cells treated with antibiotics. In those cases, the use of a control where only the antibiotic resistance is expressed can prove valuable in assessing the specificity of the cellular response to the expression of a protein of interest.

Application(s)

- Negative control in experiments involving transduction.
- Generate cell pools following puromycin selection, to validate the specificity of the effects observed by expression of a protein of interest.

Formulation

The lentivirus particles were produced in HEK293T cells in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Size and 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.

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.

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Firefly Luciferase Lentivirus (EF1A Promoter/Puromycin)	BPS Bioscience #78740-P
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
96-well tissue culture-treated assay plates	
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using the Expression Negative Control 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 the appropriate antibiotic prior to carrying out the reporter assays.

Day 1:

1. Harvest HEK293 cells from culture and seed cells at a density of 5,000-10,000 cells per well into a clear-bottom 96-well microplate in 100 µl of Thaw Medium 1.

2. Incubate the cells at 37°C with 5% CO_2 overnight.

Day 2:

1. Add 1 µl of Expression Negative Control Lentivirus into each control well.

2. Add adequate amount of Firefly Luciferase Lentivirus (EF1A Promoter/Puromycin) (or lentivirus expressing the protein of interest) to the appropriate wells.

3. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each well at a final concentration of 5 µg/ml.

4. Gently swirl the plate to mix.

5. Incubate the plate at 37°C with 5% CO_2 overnight.

   Note: Alternatively, cell seeding and transduction can be performed at the same time.

Day 3:

1. Remove the medium containing the lentivirus from the wells.

2. Add 100 µl of fresh Thaw Medium 1 to each well.

   Note: If neither the polybrene nor the lentiviruses 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.

Day 4-5:

1. Approximately 48-72 hours after transduction, if testing luciferase activity add 100 µl of ONE-Step™ Luciferase reagent to cells to measure the luciferase activity.

Notes

To generate an Expression Negative Control 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 killing curve, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, following by clonal selection.

Figures and Validation Data

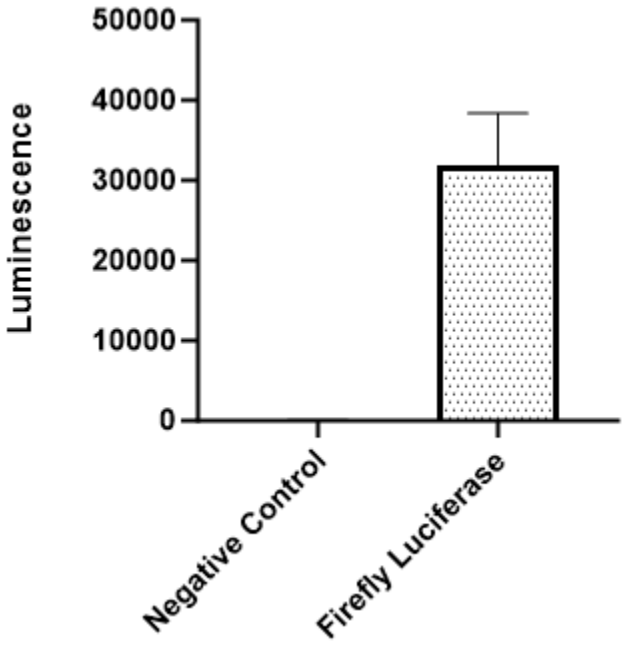

Figure 2. Luciferase activity in HEK293 cells transduced with Expression Negative Control Lentivirus and Firefly Luciferase Lentivirus.
10,000 HEK293 cells were infected with 0.5 µl of Expression Negative Control Lentivirus or Firefly Luciferase Lentivirus (EF1A Promoter/Puromycin). 48 hours post-transduction, luciferase activity was measured with ONE-Step™ Luciferase Assay System.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide
Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For further questions, please email support@bpsbioscience.com.

References
Ryu A., *et al.*, 2017 *Scientific Reports* 7:7533.

Related Products

Products	*Catalog #*	*Size*
Firefly Luciferase-eGFP Lentivirus (G418 or Puromycin)	79980	500 µl x 2
Enhanced GFP Lentivirus (G418, Hygromycin and Puromycin)	78639	500 µl x 2
mCherry Lentivirus (Hygromycin or Puromycin)	78932	500 µl x 2
Firefly Luciferase- mCherry Lentivirus (Hygromycin or Puromycin)	78933	500 µl x 2

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