Description

The Early growth response 1 (EGR1, also known as ZNF268 or NGFi-A) Promoter Luciferase Reporter Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a firefly luciferase gene driven by the human EGR1 promoter (~1.3 kb, Figure 1). After transduction, activation of the EGR1 promoter in the target cells can be monitored by measuring the luciferase activity.

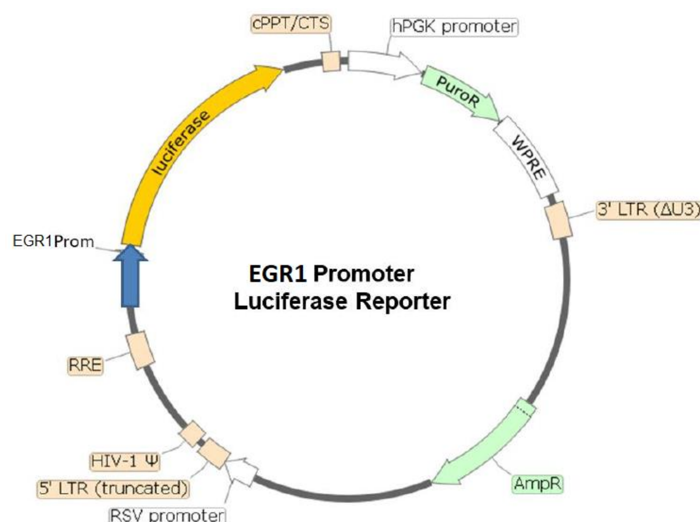

Figure 1. Schematic of the lenti-vector used to generate the EGR1 Promoter Luciferase Reporter Lentivirus.

Applications

- Screen for activators or inhibitors of EGR1 promoter activity in transduced cells.
- Generation of an EGR1 promoter luciferase reporter stable cell line (puromycin resistant).

Background

Early growth response factor 1 (EGR1) is a member of the EGR family and an important transcription factor. It contains an activation regulatory region, a repressive regulatory region, and three Cys2-His2 subclass zinc finger structures, all recognizing and binding target genes and regulating their transcription. Widely expressed in many different cell types, EGR1 is important in many physiological processes, including cell proliferation and inflammatory factors. EGR1 is a key molecule implicated in many signaling pathways and can act as a tumor suppressor to help monitor DNA damage. It can also promote tumor cell apoptosis and enhance the anticancer effects of radiotherapy and chemotherapy. Alternatively, increased EGR1 expression in hypoxic microenvironments will maintain tumor cell survival and proliferation. Researching the mechanisms of action of EGR1 can help study potential cancer therapies and new cancer treatment studies.

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 will be provided with each shipment.

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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 the third generation 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 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 the lentivirus but are necessary to follow the protocol described in the "Validation Data" section. Media, reagents, and luciferase assay buffers used at BPS Bioscience are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
Phorbol 12-Myristate 13-Acetate (PMA)	LC Laboratories #P-1680
Recombinant human epidermal growth factor (EGF)	BPS Bioscience #90201
HEK293 growth medium or use Thaw Medium 1	BPS Bioscience #60187
Assay Medium 1D	BPS Bioscience #78745
Polybrene	Millipore #TR-1003-G

Assay Protocol

The following protocol is a general guideline for transducing HepG2 cells using EGR1 promoter luciferase reporter 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 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 carrying out the reporter assays.

1. Day 1: Seed HepG2 cells at a density of 5,000-10,000 cells per well into white, clear bottom 96-well cell culture plate in 90 µl of Thaw Medium 1 (BPS Bioscience #60187).

   To each well, add 2 µl of EGR1 Promoter Luciferase reporter lentivirus. Optional: 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 48 hours.

2. Day 3: Remove the medium containing the lentivirus from the wells.

   Add 100 µl of Assay Medium 1D (BPS Bioscience #78745) containing EGF or PMA to induce EGR1.
   Add 100 µl of Assay Medium 1D to "control untreated" wells.

3. Incubate the plate at 37°C with 5% CO_2 for 6 hours.

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4. Prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Validation Data

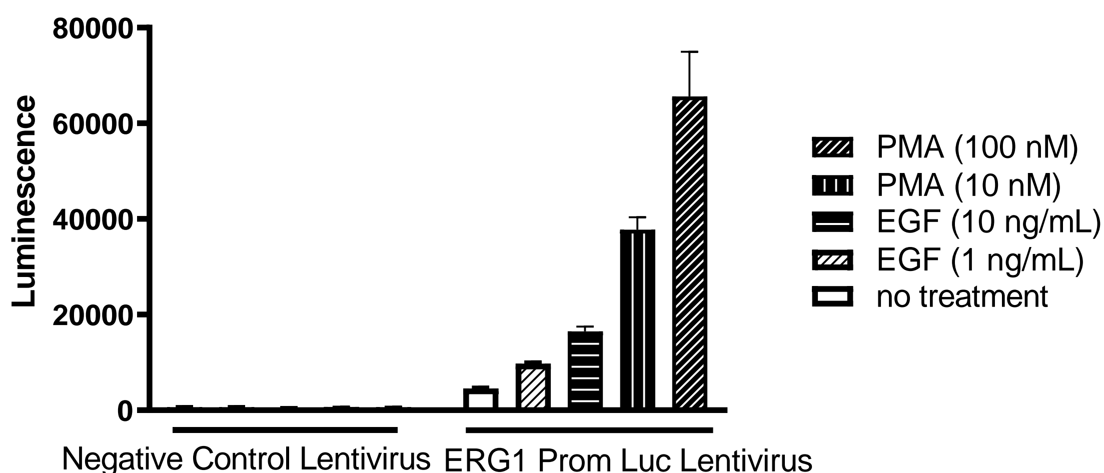

Figure 2. EGR1 Promoter Luciferase Reporter stimulated by EGF and PMA in HepG2 cells.
Approximately 10,000 HepG2 cells/well were transduced with 100,000 TU/well of negative control lentivirus (BPS Bioscience #79578) or EGR1 Promoter Luciferase reporter lentivirus. After 48 hours of transduction, the growth medium was changed to assay medium 1D, and the cells were treated with EGF or PMA for ~6 hours. The results are shown as the raw luminescence reading.

Notes

1. To generate an ERG1 promoter luciferase 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.
2. The following Lentivirus Reporter Controls are available from BPS Bioscience to meet your experimental needs:
    a. Negative Control Luciferase Lentivirus (BPS Bioscience #79578): Ready-to-transduce lentiviral particles expressing firefly luciferase under the control of a minimal promoter. The negative control is important to establish the specificity of any treatment and to determine the background reporter activity.
    b. Renilla Luciferase Lentivirus (BPS Bioscience #79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the CMV promoter. The RLuc lentivirus can serve as an internal control to overcome sample-to-sample variability when performing dual-luciferase reporter assays.
    c. Firefly Luciferase Lentivirus (BPS Bioscience #79692-G, #79692-H, #79692-P): Ready-to-transduce lentiviral particles expressing firefly luciferase under the CMV promoter. It serves as a positive control for transduction optimization studies.

Reading Luminescence
Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay.

References
Wang, B., et. al,. The Role of the Transcription Factor EGR1 in Cancer. Frontiers in Oncology. (2021) 11.

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*
XRE Luciferase Reporter Lentivirus	78672	500 µl x 2
p53Luciferase Reporter Lentivirus	78666	500 µl x 2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x 2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x 2

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