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Lieferung & Zahlungsart

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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Hypoxia Response Element (HRE) Luciferase Reporter Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce most types of mammalian cells, including primary and non-dividing cells. The particles contain a firefly luciferase gene driven by four copies of a hypoxia response elements (HRE) located upstream of the minimal TATA promoter (Figure 1) and an antibiotic selection gene (puromycin) for the selection of stable clones. After transduction, the induction of hypoxia in the target cells can be monitored by measuring the luciferase activity.

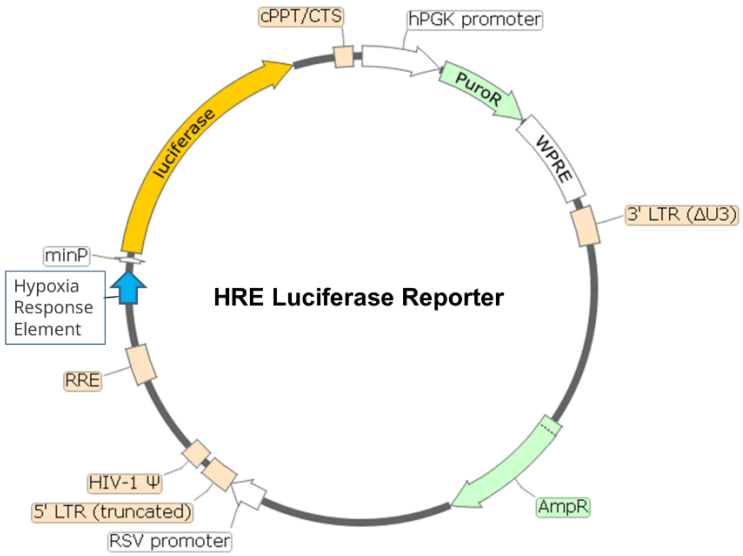

Figure 1. Schematic of the lenti-vector used to generate the HRE luciferase reporter lentivirus.

Background

Hypoxia occurs in solid tumors as a result of poor vascularization within the core of the tumor. It is a driver of tumor progression and resistance to therapy through adaptive responses. Hypoxia response elements (HREs) are transcription factor binding sites within the promoters of various genes regulated by hypoxia-inducible factors (HIFs). As oxygen becomes rate limiting, HIFs form heterodimers that recognize cognate HREs, thus activating the transcription of genes involved in cell proliferation, metastasis, and angiogenesis. HIF activation in many types of cancer correlates with poor outcomes.

Application(s)

- Screen for activators or inhibitors of hypoxia-related signaling pathways
- Generate an HRE luciferase reporter stable cell line (puromycin resistant) following puromycin selection and limiting dilution

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.

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 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 Used for Validation but Not Supplied

These materials are not supplied with this lentivirus but were used to follow the protocol described in the "Transduction Protocol" 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
HEK293	ATCC #CCL-1573
Thaw Medium 1	BPS Bioscience #60187
96-well tissue culture, clear-bottom, white plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Transduction Protocol

The following protocol was used to transduce HEK293 cells using the HRE 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 HEK293 cells at a density of 5,000-10,000 cells per well into white, clear bottom 96-well microplate in 90 µl of Thaw Medium 1 (BPS Bioscience #60187).

   To each well, add 2 µl of HRE luciferase reporter lentivirus.

   Optional: Add polybrene to each well to 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 Thaw Medium 1 containing the tested compounds to "Stimulated" cells.

- Add 100 µl of Thaw Medium 1 to the "Control untreated" cells (to determine the luminescence from the transduced HEK293 cells).

- Add 100 µl of Thaw Medium 1 to cell-free control wells (for determine the background luminescence).

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

3. Day 4: Perform the ONE-Step™ Luciferase assay (BPS Bioscience #60690) as per recommended protocol (100 µl/well). Incubate the plate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Validation Data

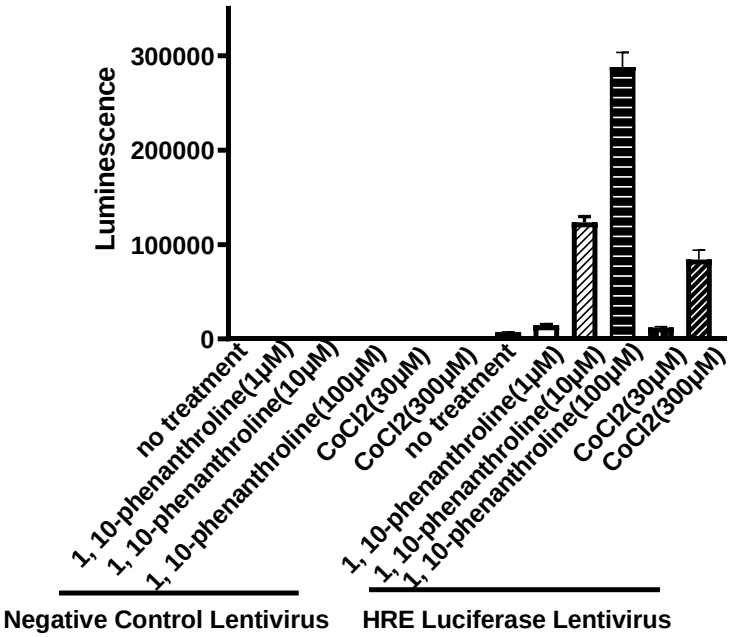

Figure 2. Activation of HRE luciferase reporter activity in HEK293 cells.
Approximately 8,000 HEK293 cells/well were transduced with 40,000 TU/well HRE Luciferase Reporter Lentivirus. After 48 hours of transduction, the medium was changed to Thaw Medium 1 containing 1, 10-phananthroline (Sigma #131377) or $CoCl_2$ (Sigma #C8661) at the indicated concentrations. The plate was incubated at 37°C with 5% CO_2 overnight. Results are shown as raw luminescence signal. The Negative Control Luciferase Lentivirus (BPS Bioscience #79578) was transduced in parallel.

Notes

1. To generate a HRE 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. To determine the concentration of puromycin needed for your cell line, perform a kill curve (for more information, visit our Resources page, frequently asked questions: what is a kill curve?).

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 treatments 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 Renilla Luciferase 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.

Reference

Doran *et. al,.* Hypoxia activates constitutive luciferase reporter constructs. *Biochimie*. (2011) **93(2)**: 361-368.

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
p53 Luciferase Reporter Lentivirus	78666	500 µl x 2
ARE Reporter HepG2 Cell line (Nrf2 Antioxidant Pathway)	60513	2 vials