Description

The UAS (Upstream Activation Sequence) Luciferase Reporter Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a firefly luciferase gene driven by a multimerized GAL4 upstream activation sequence (UAS) located upstream of the minimal TATA promoter (Figure 1) and an antibiotic selection gene (puromycin) for the selection of stable clones. After transduction, the UAS-controlled signaling pathway in the target cells can be monitored by measuring the luciferase activity.

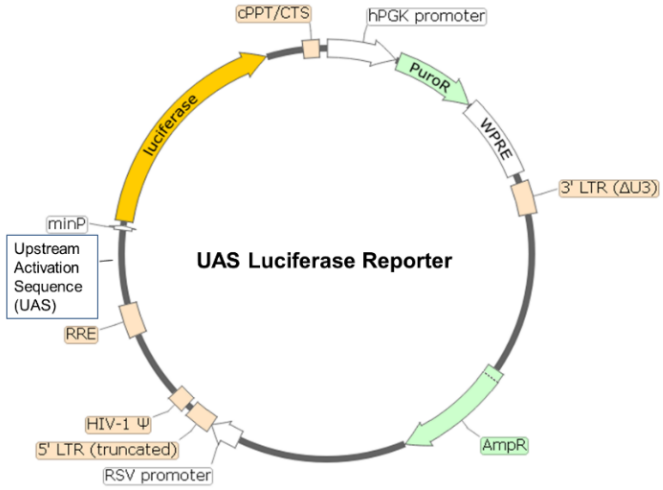

Figure 1. Schematic of the lenti-vector used to generate the UAS luciferase reporter lentivirus.

Background

GAL4/UAS is a binary gene expression system used for modeling expression vectors or transgenic animals with GAL4 and UAS constructs. UAS is a regulatory sequence critical to induction and the increased expression of a nearby gene of interest. GAL4 binds to UAS to activate transcription from a basal promoter located downstream of the activation sequence. Gal4/UAS is a highly advantageous system that can induce gene expression at a desired time and at higher levels than endogenous tissue-specific promoters. It can link a portion of a nuclear receptor to a reporter system to study ligand binding or to screen small molecules.

GAL4 DBD-GR lentivirus (BPS Bioscience #78632), expresses a fusion protein where the glucocorticoid receptor (GR) ligand binding domain is fused to the DNA binding domain of GAL4 (GAL4 DBD). After co-transduction of UAS luciferase reporter lentivirus and GAL4 DBD-GR lentivirus. The glucocorticoid-induced activation of the glucocorticoid receptor can be monitored by measuring the luciferase activity.

The glucocorticoid signaling pathway plays an important role in development, fluid homeostasis, cognition, immune response and metabolism. Glucocorticoids are a class of steroid hormones that bind to the glucocorticoid receptor, causing it to translocate to the nucleus. Upon translocation, the receptor can regulate the transcription of many genes, including those that regulate glucose metabolism and inflammatory responses.

Application(s)

- In combination with GAL4 DBD-GR lentivirus (BPS Bioscience #78632) to monitor glucocorticoid signaling pathway activity.
- Generate a UAS Luciferase Reporter stable cell line (puromycin resistant)

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 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 the 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, 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 #CRL-1573
Thaw Medium 1	BPS Bioscience #60187
GAL4 DBD-GR Lentivirus	BPS Bioscience #78632
Dexamethasone	Sigma #D4902
Polybrene Infection / Transfection Reagent	Millipore #TR-1003-G
96-well Clear Bottom White Polystyrene TC-treated	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using the UAS 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 2.5 µl of UAS luciferase reporter lentivirus and 2.5 µl of GAL4 DBD-GR 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 24 hours.

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

   Add 100 µl of Thaw Medium 1 containing 2 µM dexamethasone into the stimulated wells.

   Add 100 µl of Thaw Medium 1 to the control untreated wells.

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

   Incubate the plate at 37°C with 5% CO_2 for 24 hours.

3. Day 3: 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

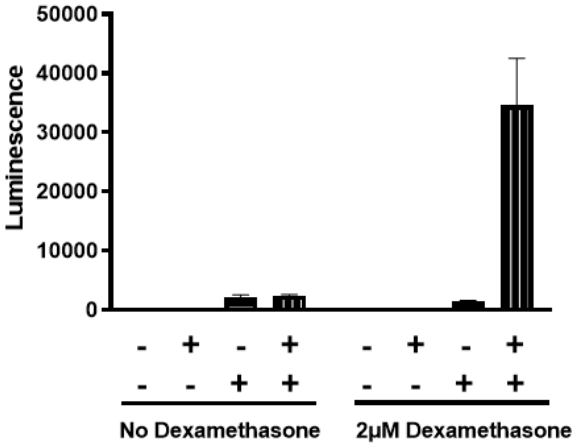

Figure 2. Dexamethasone-induced activation of the UAS luciferase reporter with GAL4 DBD-GR. Approximately 10,000 HEK293 cells/well were co-transduced with 50,000 TU/well UAS Luciferase Reporter Lentivirus and 50,000 TU/well GAL4 DBD-GR Lentivirus. After 24 hours of transduction, the medium was changed to fresh Thaw Medium 1 and the cells were stimulated with 2 µM dexamethasone for 24 hours. Results are shown as the raw luminescence reading. Cells were also transduced in parallel with GAL4 DBD-GR or UAS Luciferase lentivirus only as controls.

Notes

1. To generate the UAS 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 Luciferase 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-P, #79565-G): 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.

References

1. Paguio A, et al. “*Improved Dual-Luciferase Reporter Assays for Nuclear Receptors*.” Curr Chem Genomics. **4: 43-49,** 2010
2. Yamada, Mayumi, et al. “Optimization of Light-Inducible Gal4/UAS Gene Expression System in Mammalian Cells.” iScience, **vol. 23, no. 9,** 2020, p. 101506.

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*
Negative Control Luciferase Lentivirus	79578	500 µl x 2
Renilla Luciferase Lentivirus	79565	500 µl x 2
Firefly Luciferase Lentivirus	79692	500 µl x 2
GAL4 DBD-GR Lentivirus	78632	500 µl x 2
GAL4 Reporter Kit (Glucocorticoid Receptor Pathway)	60522	500 reactions
GR-GAL4 Reporter (Luc)-HEK293 cell line	60655	2 vials