Description

GAL4 DBD-GR (GAL4 DNA binding domain-glucocorticoid receptor) lentivirus 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 express a fusion protein in which the glucocorticoid receptor (GR) ligand binding domain is fused to the DNA binding domain of GAL4 (GAL4 DBD) (Figure 1). After co-transduction of GAL4 DBD-GR lentivirus and GAL4-responsive UAS luciferase reporter lentivirus (BPS Bioscience #78631), the glucocorticoid-induced activation of the glucocorticoid receptor can be monitored by measuring the luciferase activity.

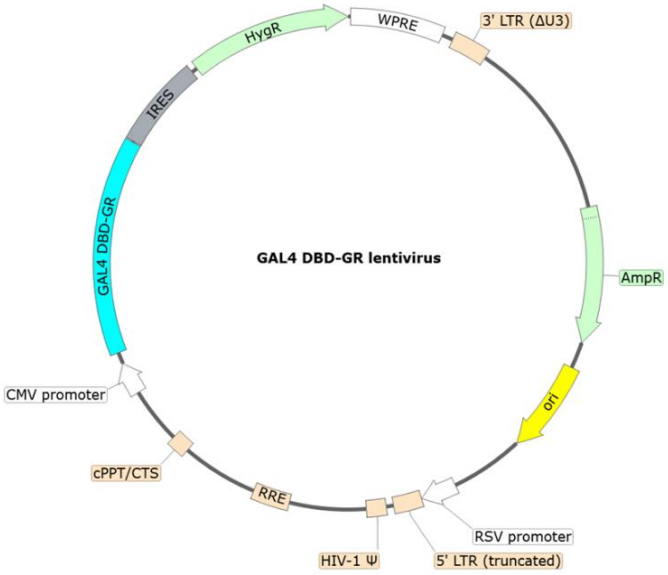

Figure 1. Schematic of the lenti-vector used to generate the GAL4 DBD-GR Lentivirus.

Background

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 UAS Luciferase reporter lentivirus (BPS Bioscience #78631), to monitor glucocorticoid signaling pathway activity.
- Generate a GAL4 DBD-GR stable cell line (Hygromycin 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 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, 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
UAS luciferase reporter lentivirus	BPS Bioscience #78631
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 GAL4 DBD-GR Lentivirus and 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 UAS luciferase reporter (puromycin) and GAL4 DBD-GR (hygromycin) 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

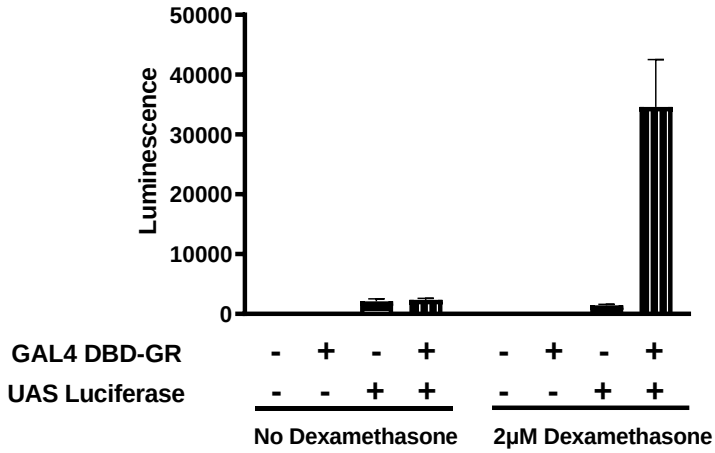

Figure 2. Dexamethasone-induced activation of the UAS luciferase reporter with GAL4 DBD-GR. Approximately 10,000 HEK293 cells/well were co-transduced with 50,000 TU/well UAS Luciferase Reporter Lentivirus and 50,000 TU/well GAL4 DBD-GR. After 24 hours of transduction, the medium was changed to fresh 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 with GAL4 DBD-GR or UAS Luciferase lentivirus only as controls.

Notes

To generate a GAL4 DBD-GR stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of hygromycin (as pre-determined from a killing curve) for antibiotics selection of transduced cells.

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

Paguio A, *et al*. (2010) Improved Dual-Luciferase Reporter Assays for Nuclear Receptors. Curr Chem Genomics. 4: 43-49.

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
UAS Luciferase Reporter Lentivirus	78631	500 µl x 2
GAL4 Reporter Kit (Glucocorticoid Receptor Pathway)	60522	500 reactions
GR-GAL4 Reporter (Luc)-HEK293 cell line	60655	2 vials
GAL4 Reporter (Luc)-HEK293 Recombinant Cell Line	60656	2 vials