Description

The STAT6 Luciferase Reporter Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce most mammalian cells, including primary and non-dividing cells. These viruses contain a firefly luciferase reporter driven by multiple copies of STAT6 (signal transducer and activator of transcription 6) responsive elements located upstream of the minimal TATA promoter. The lentiviruses also contain a puromycin selection marker (Figure 1).

After transduction, the cellular STAT6 signaling pathway can be monitored by measuring the luciferase activity.

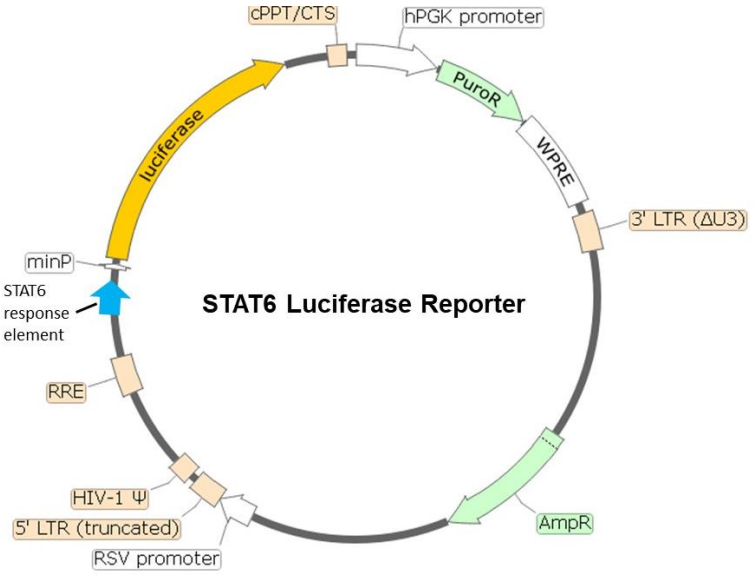

Figure 1. Schematic of the lenti-vector used to generate the STAT6 Luciferase Reporter Lentivirus.

Background

STAT6 (signal transducer and activator of transcription 6) is a member of the STAT family of proteins which activate gene expression by relaying signals from cytokines that bind to their receptors on the cell surface to the nucleus. STAT6 is mainly activated by IL-4 (interleukin-4) and IL-13, via phosphorylation by JAKs (Janus kinases). Once phosphorylated, STAT6 forms a homodimer that translocate to the nucleus and binds to DNA via the DNA-binding domain, activating gene transcription. STAT6 signaling is involved in T-helper type 2 (Th2) cell development and differentiation, macrophage activation, and it impacts the levels of CD20 on the surface of B lymphocytes. Gene fusions products of STAT6 with NGFI-A-binding protein 2 (NAB2) are responsible for fibrous tumors. Dysfunction of the STAT6 pathway leads to allergic reactions (asthma, atopic dermatitis, food allergies) and cancer (Hodgkin, central nervous system and follicular lymphoma). STAT6 can serve as a diagnostic and prognostic tool. Inhibitors have been shown to reduce acute inflammation in animal models, and future developments may lead to new therapeutical tools for STAT6 related pathologies.

Application

- Screen for activators or inhibitors of the STAT6 signaling pathway.
- Generate STAT6 Luciferase Reporter cell pools or stable cell lines by puromycin selection.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture 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 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 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
HepG2 Cells	ATCC #HB-8065
Thaw Medium 1	BPS Bioscience #60187
Recombinant Human IL-4 Protein	R&D Systems# BT-004-010
96-well tissue culture, clear-bottom, white plate	Corning #3610
One-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Media Required for the Proposed Assay

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin

Assay Protocol

The following protocol is a general guideline for transducing HepG2 cells using the STAT6 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, it may be necessary to select the cells expressing the reporter gene with puromycin, creating a cell pool or stable cell line, prior to carrying out the reporter assays.

Day 1:

1. Seed HepG2 cells at a density of 5,000-10,000 cells per well in 90 µl of Thaw Medium 1 into a white, clear bottom 96-well microplate.

2. To each well, add 2 µl of STAT6 Luciferase Reporter lentivirus.

   Optional: Add polybrene to each well to a final concentration of 5 µg/ml.

3. Gently swirl the plate to mix.

4. Incubate the plate at 37°C with 5% CO_2 for 48-66 hours.

Day 3 or 4:

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

2. Add 100 µl of Thaw Medium 1 containing the compounds being tested to the "Stimulated" wells.

3. Add 100 µl of Thaw Medium 1 to the "Control Untreated" wells (to determine the unstimulated luminescence from the transduced HepG2 cells).

4. Add 100 µl of Thaw medium 1 to the "Cell-Free Control" wells (to determine the background luminescence).

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

6. Add 100 µl/well of ONE-Step™ Luciferase assay reagent.

7. Incubate the plate at Room Temperature (RT) for ~15 to 30 minutes.

8. Measure luminescence using a luminometer.

Validation Data

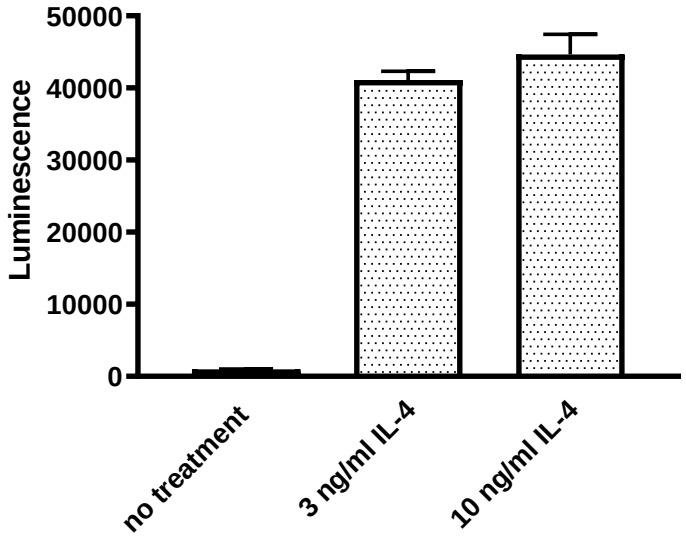

Figure 2. Activation of STAT6 luciferase reporter activity in HepG2 cells transduced with STAT6 Luciferase Reporter Lentivirus.
Approximately 10,000 HepG2 cells/well were transduced with 40,000 TU/well of STAT6 Luciferase Reporter Lentivirus. After 48 hours of transduction, cells were treated with various concentrations of IL-4 for 6 hours. Luciferase activity was measured using ONE-Step™ Luciferase. Luciferase activity was detected only in the presence of IL-4. Results are shown as the raw luminescence reading.

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

Notes

1. To generate a STAT6 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 (as pre-determined from a killing curve, FAQs (bpsbioscience.com) for antibiotic selection of transduced cells, followed by clonal selection.
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. This 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 control of a CMV promoter. The Renilla luciferase lentiviruses 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 control of a 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

Kim M., *et al.*, 2018 *Cell Physiol Biochem* 45(5):1863-1877.
Hu X., *et al.*, 2021 *Signal Transduction and Targeted Therapy* 6: 402.
Karpathiou G., *et al.*, 2021 *Pathol Res Pract.* 223:153477.

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
Firefly Luciferase Lentivirus	79692	500 µl x 2
Renilla Luciferase Lentivirus	79565	500 µl x 2