

Description

The STAT3 Renilla Luciferase Reporter Lentivirus (Zeocin) 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 Renilla luciferase reporter under the control of STAT3 (Signal Transducer and Activator of Transcription 3)-responsive element located upstream of the minimal TATA promoter (Figure 1). After transduction, activation of the STAT3 signaling pathway in the target cells can be monitored by measuring renilla luciferase activity.

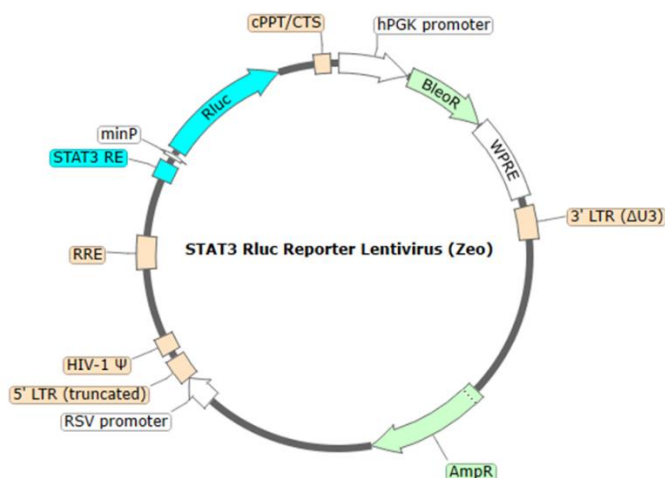


Figure 1. Schematic of the lenti-vector used to generate the STAT3 Renilla Luciferase Reporter Lentivirus (Zeocin).

Background

STAT3 (Signal Transducer and Activator of Transcription 3) is a transcription factor expressed in diverse cell types. STAT3 is tyrosine phosphorylated by JAK (Janus Kinases) and translocated to the nucleus. While it was initially described as an acute phase response factor in the context of IL-6 (interleukin 6) signaling, STAT3 has since been identified as a downstream effector of many cytokines. STAT3-mediated IL-6 signaling in macrophages plays a role in their differentiation and proliferation. The JAK-STAT signaling pathway is involved in both cancer and autoimmune diseases and has been an attractive target for drug discovery in immunological diseases and cancer. The presence of Renilla luciferase allows for easy assay readouts.

Application

- Screen for activators or inhibitors of STAT3 signaling pathway in transduced target cells.
- Generate a stable cell line expressing STAT3 renilla luciferase following zeocin 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 and produced at higher titers by special request, for an additional fee.

Size and Titer

Two vials (500 μ l x 2) of lentivirus at a titer $\geq 10^7$ 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 for up to 12 months from date of receipt. 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 the Lentivirus but are necessary to perform the cellular assays described below. BPS Bioscience’s reagents are validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
IL-6 (Interleukin 6) Recombinant (Human)	BPS Bioscience #83844
96-well tissue culture-treated white clear-bottom plate	Corning #3610
TWO-Step™ Luciferase Assay System	BPS Bioscience #60683
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 Cell Culture and Functional Cellular 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 HEK293 cells using STAT3 Renilla Luciferase Reporter Lentivirus (Zeocin). The optimal transduction conditions (e.g. MOI, concentration of Lenti-Fuse™ Polybrene Viral Transduction Enhancer, time of assay development) should be optimized according to the cell type and the assay requirement. In most cell types, the expression of the reporter 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 zeocin prior to carrying out the reporter assays.
- The assay should include “Stimulated”, “Cell-Free Control” and “Untreated Control” conditions.

Day 1:

1. Seed HEK293 cells at a density of ~8,000 cells per well in 90 μ l of Thaw Medium 1 into a white, clear bottom 96-well cell culture plate. Leave a few empty wells as “Cell-Free Control” (background luminescence).
2. To each well, add 5 μ l of STAT3 Renilla Luciferase Reporter lentiviruses.
3. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each well at a final concentration of 5 μ g/ml.
4. Gently swirl the plate to mix.
5. Incubate the plate at 37°C with 5% CO₂ for 48 hours.

Note: Alternatively, seeding cells and the transduction can be performed on the same day.

Day 3:

1. 48 hours after transduction, remove the medium containing the lentiviruses from the wells.
2. Prepare the compound of interest in Thaw Medium 1 at the final concentration to be tested (100 μ l/ well).
3. Add 100 μ l of compound to the “Stimulated” wells.
4. Add 100 μ l of Thaw Medium 1 to the “Untreated Control” wells (to determine the luminescence from the transduced, unstimulated HEK293 cells).
5. Add 100 μ l of Thaw Medium 1 to the “Cell-Free Control” wells (to determine the background luminescence).
6. Incubate the plate at 37°C with 5% CO₂ for 24 hours.

Day 4:

1. To measure Renilla Luciferase activity, use TWO-Step Luciferase (Firefly & Renilla) Assay System (#60683) following the recommended protocol.
2. Measure luminescence using a luminometer.

Notes

To generate a STAT3 renilla luciferase reporter cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of zeocin as pre-determined from a killing curve (visit <https://bpsbioscience.com/kill-curve-protocol>), for antibiotic selection of transduced cells, followed by clonal selection.

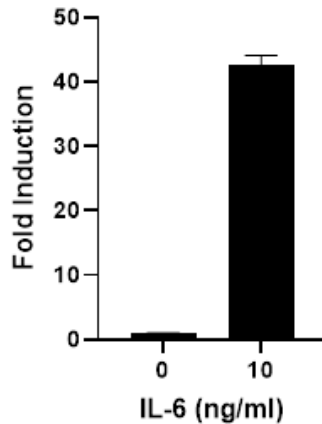
Validation Data

Figure 2. STAT3 renilla luciferase reporter activity is stimulated by IL-6 in HEK293 cells transduced with STAT3 Renilla Luciferase Reporter Lentivirus (Zeocin).

Approximately 10,000 HEK293 cells/well were transduced with 30,000 TU/well STAT3 Renilla Luciferase Reporter Lentivirus. 48 hours post-transduction, the cells were treated with IL-6 for 24 hours. The results are shown as fold induction of luciferase reporter expression compared to control cells without IL-6 treatment.

Data shown is representative.

References

Tian S., *et al.*, 1994 *Blood*. 84(6):1760-1764.
 Zhong Z., *et al.*, 1994 *Science*. 264(5155):95-98.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
STAT3 mCherry Reporter Lentivirus	82375	500 µL X2
STAT3 eGFP Reporter Lentivirus	78197	500 µL X2
STAT3 Luciferase Reporter Lentivirus	79744	500 µL x 2
STAT3 Luciferase Reporter THP-1 Cell Line	78498	2 vials
STAT3 Reporter Jurkat Cell Line	78497	2 vials
STAT3 Luciferase Reporter HEK293 Cell Line	79800-P	2 vials

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