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Data Sheet

STAT5 Luciferase Reporter Lentivirus

Catalog #: 79745

Product Description
The STAT5 Luciferase Reporter Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a firefly luciferase gene under the control of STAT5-responsive element located upstream of the minimal TATA promoter (Figure 1). After transduction, activation of the STAT5 signaling pathway in the target cells can be monitored by measuring the luciferase activity.

Applications
- Screen for activators or inhibitors of STAT5 signaling pathway in the transduced target cells
- Generation of STAT5 luciferase reporter stable cell line

Formulation
The lentiviruses were produced from HEK293T cells and are provided in medium containing 90% DMEM + 10% FBS.

Titer
Two vials (500 µl x 2) of STAT5 luciferase reporter lentivirus at a titer ≥1 x 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 virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety
The lentiviruses are produced with the second generation 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.

Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

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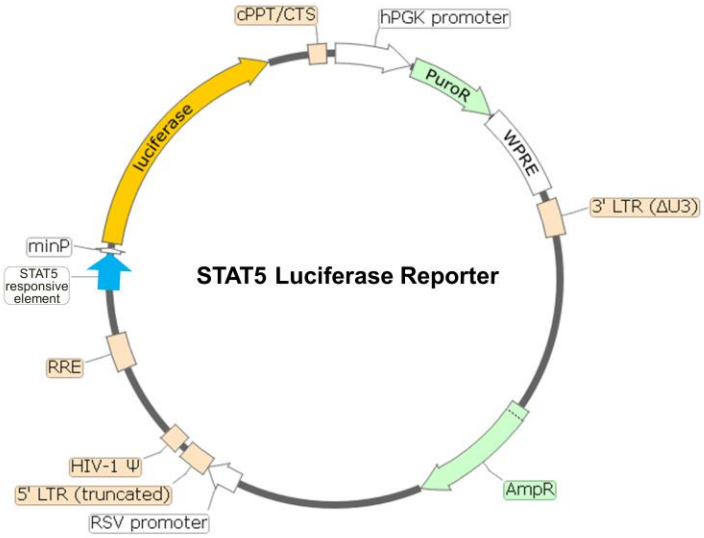

Figure 1. Schematic of the lenti-vector used to generate the STAT5 luciferase reporter lentivirus

Materials Required but Not Supplied

- mIL-3 (R&D Systems #403-ML-010)
- BaF3 growth medium: Thaw Medium 8 (BPS Bioscience, #79652) supplemented with 5 ng/mL mIL-3
- Assay medium: Thaw Medium 8 (BPS Bioscience, #79652)
- Polybrene (Millipore, #TR-1003-G)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- One-Step luciferase assay system (BPS Bioscience, #60690)
- Luminometer

Assay Protocol

The following protocol is a general guideline for transducing BaF3 cells using STAT5 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. Harvest BaF3 cells by centrifugation and resuspend the cells in fresh growth medium. Dilute the cells to 0.4 x 10^6 /ml in growth medium. Mix 1 ml of the BaF3 cells and 100 µl of STAT5 luciferase reporter lentivirus in a 1.5-ml Eppendorf tube. Add polybrene to a final

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concentration of 8 µg/ml. Gently mix and incubate the virus with the BaF3 cells for 20 minutes at room temperature in the tissue culture hood.

2. Centrifuge the virus/cells mixture for 30 minutes at 800 x g at 32°C. Remove the virus containing medium and resuspend the cell pellet in 2 ml of fresh BaF3 growth medium. Transfer the cells into one well in a 6-well plate. Incubate the plate at 37°C with 5% CO_2 for 48 hours. The transduced BaF3 cells are ready for assay development.

3. Harvest the cells and wash the BaF3 cells once with assay medium. Resuspend the BaF3 cells into 1200 µl assay medium. Add 90 µl of the cells to each well of the 96-well plate.

4. Prepare diluted mIL-3 in assay medium. Add 10 µl of mIL-3 into the mIL-3 stimulated cells. Add 10 µl of assay medium to the unstimulated control wells (for measuring the uninduced level of STAT5 reporter activity).

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

6. Prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Important Notes:

1. To generate the STAT5 luciferase reporter stable cell line, remove the growth medium 48 hours after transduction and replaced it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.

2. The following Lenti Reporter Controls are also available from BPS Bioscience to meet your experimental needs:

1) Negative Control 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.

2) Renilla Luciferase (Rluc) Lentivirus (BPS Bioscience, #79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the CMV promoter. The RLuc lentivirus can serve as an internal control to overcome sample-to-sample variability when performing dual-luciferase reporter assays.

3) Firefly Luciferase (Fluc) Lentivirus (BPS Bioscience, #79692-G, #79692-H, #79692-P): Ready-to-transduce lentiviral particles expressing firefly luciferase under the CMV promoter. The Fluc lentivirus can serve as a positive control for transduction optimization studies.

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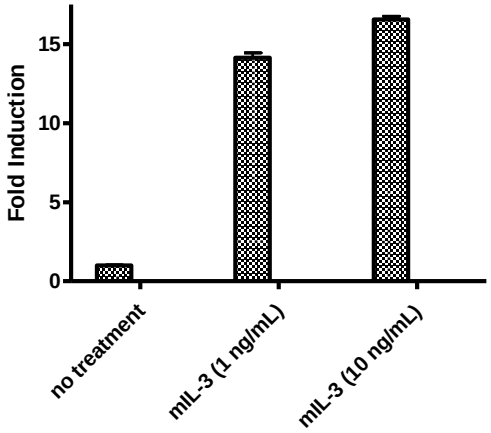

Figure 2. STAT5 luciferase reporter activity stimulated by mIL-3 in BaF3 cells. Approximately 40,000 BaF3 cells/well were transduced with 100,000 TU/well STAT5 luciferase reporter lentivirus. After 48 hours of transduction, the medium was changed to assay medium, and the cells were treated with mIL-3 for 5 hours. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without mIL-3 treatment.

Related Products

Product	Cat. #	Size
NF-κB Luciferase Reporter Lentivirus	79564	500 µl x2
CRE Luciferase Reporter Lentivirus	79580	500 µl x2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x2
TCF/LEF Luciferase Reporter Lentivirus	79787	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (G418)	79692-G	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Hygromycin)	79692-H	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Puromycin)	79692-P	500 µl x2
STAT5 Reporter (Luc)-BaF3 cell line	79772	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Dual Luciferase (Firefly-Renilla) Assay System	60683	10 ml
Thaw Medium 8	79652	100 ml

References

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