Description

The NFAT Luciferase-eGFP 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 and eGFP cassette driven by the NFAT response element located upstream of the minimal TATA promoter (Figure 1) and a puromycin selection gene to generate stable clones. After transduction, activation of the NFAT signaling pathway in the target cells can be monitored by measuring the luciferase activity or eGFP expression.

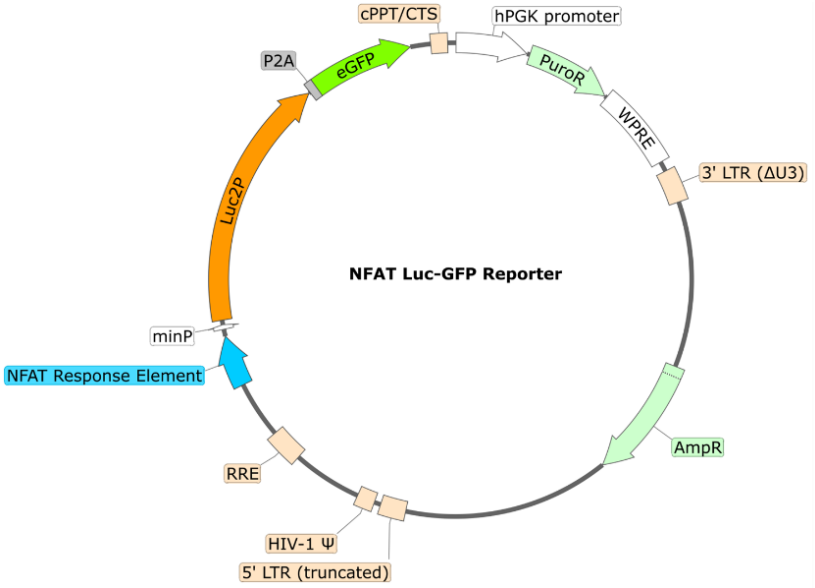

Figure 1. Schematic of the lenti-vector used to generate the NFAT luciferase-eGFP reporter lentivirus (puromycin).

Background

NFAT (Nuclear factor of activated T-cells) is a family of transcription factors that has an important function in immune responses, for example by inducing the expression of various cytokines (such as IL-2-3-4 and TNF-alpha) in T cells. Members of the NFAT family have been found in many tissue types including heart, skeletal muscle and brain cells. Through their role in the immune system, NFATs are involved in inflammation and these transcription factors are considered promising therapeutic targets for a variety of diseases.

NFAT is regulated by Ca2+ and the Ca2+/calmodulin-dependent serine phosphatase, calcineurin. NFAT proteins are phosphorylated and reside in the cytoplasm in resting cells; upon stimulation, they are dephosphorylated by calcineurin, translocate to the nucleus, and induce gene expression.

Application(s)

- Screen for activators or inhibitors of NFAT signaling pathways in transduced target cells.
- Generate NFAT Luciferase-eGFP 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 third generation 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
Jurkat cells	ATCC #TIB-152
Anti-CD3 agonist antibody	BPS Bioscience #71274
Thaw Medium 2	BPS Bioscience #60184
Polybrene	Millipore #TR-1003-G
96-well tissue culture, clear-bottom, white plate	Corning #3610
One-Step Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Assay Protocol

The following protocol is a general guideline for transducing Jurkat cells using the NFAT luciferase-eGFP 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: Harvest the Jurkat cells by centrifugation and resuspend the cells in fresh Thaw Medium 2. Dilute the cells to a density of 2 x 10^5/ml in medium. Mix 500 µl of the Jurkat cells and 400 µl of NFAT luciferase-eGFP reporter lentivirus in a 1.5-ml Eppendorf tube (at a MOI >10).

   Add polybrene to a final concentration of 8 µg/ml. Gently mix and incubate the virus with the Jurkat cells for 20 minutes at room temperature in the tissue culture hood.

Centrifuge the virus/cell 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 Thaw Medium 2. Transfer the cells into one well of a 6-well plate. Incubate the plate at 37°C with 5% CO_2 for 48 hours. The transduced Jurkat cells are ready for assay development on day 3.

2. Day 3: Coat a cell culture-treated, clear bottom, white 96-well plate with anti-CD3 antibody diluted in PBS (Phosphate Buffer Saline) overnight. Leave a few non-coated wells to serve as negative controls.

3. Day 4: Wash all wells of the coated plate three times with PBS. The plate is ready to use.

4. Day 4: Harvest the transduced Jurkat cells (from day 1) and resuspend the cells into 900 µl of fresh Thaw Medium 2. Add 100 µl of cells to each well of the CD3 antibody-coated 96-well plate.

5. Incubate at 37°C with 5% CO_2 for 6-24 hours.

6. Prepare the ONE-Step™ Luciferase reagent per recommended protocol (100 µl/well). 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.

Validation Data

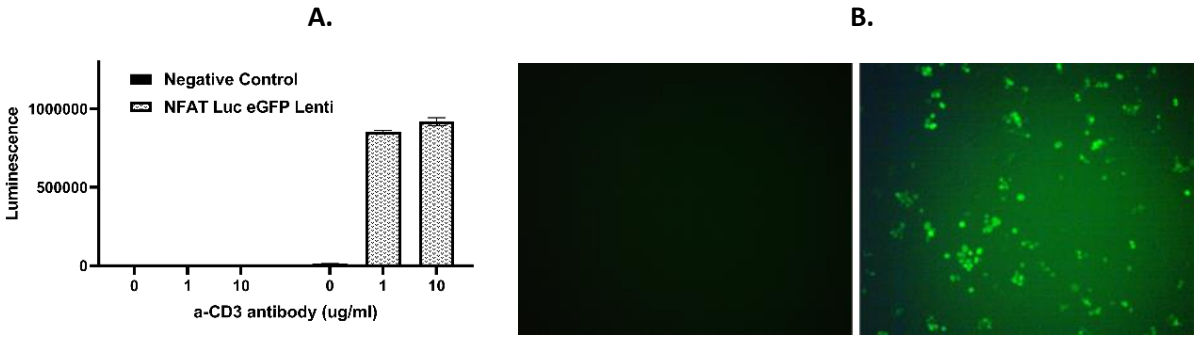

Figure 2. NFAT luciferase-eGFP reporter activity stimulated by anti-CD3 agonist antibody in Jurkat cells.
Approximately 20,000 Jurkat cells/well (96-well plate) were transduced with 300,000 TU NFAT luciferase-eGFP reporter lentiviruses. After 48 hours of transduction, the medium was changed to Thaw medium 2. Cells were stimulated by transferring them to a 96-well plate pre-coated with anti-CD3 agonist antibody for 24 hours. The non-coated wells and the negative control lentivirus (BPS Bioscience #79578) were performed in parallel as controls.
A. Results are shown as the raw luminescence reading.
B. Results are shown as eGFP expression observed under fluorescence microscopy (left, uninduced; right, induced by 10 µg/ml of anti-CD3 agonist precoated on the plate).

Notes

1. To generate the NFAT luciferase-eGFP 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 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 (G418 or Puromycin) (BPS Bioscience #79565): 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 (G418, Hygromycin and Puromycin) (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. Clipstone NA, Crabtree GR. *Nature*. 1992 Jun 25**;357(6380)**: 695-7.
2. Lyakh, L., *et al*. *Mol Cell Biol*. 1997 May;**17(5)**: 2475-84.

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*
NFAT Reporter (Luciferase) – THP-1 Cell Line	78320	500 µl x 2
NFAT eGFP Reporter Lentivirus	79922	500 µl x 2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x 2
NFAT Luciferase-RFP Reporter Lentivirus	78617	500 µl x 2