Description

NFAT eGFP Reporter 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 contain an enhanced GFP (green fluorescent protein) reporter driven by the NFAT (Nuclear Factor of Activated T Cells) response element located upstream of the minimal TATA promoter. The lentiviruses also transduce a puromycin selection gene (Figure 1). After transduction, activation of the NFAT signaling pathway in target cells can be monitored by assessing eGFP expression.

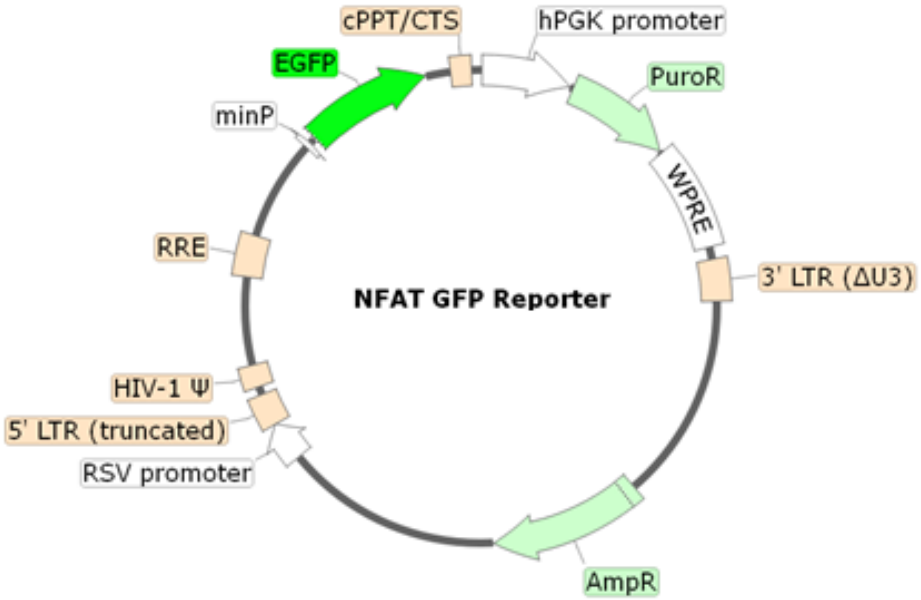

Figure 1. Schematic of the lenti-vector used to generate the NFAT eGFP Reporter Lentivirus.

Background

The NFAT (Nuclear Factor of Activated T Cells) family of transcription factors plays an important role in mediating immune responses. T cell activation through the T cell synapse results in calcium influx, which is required to facilitate actin organization and interactions at the synapse. Increased intracellular calcium levels activate the calcium-sensitive phosphatase calcineurin, which rapidly dephosphorylates the serine-rich region (SRR) and SP-repeats in NFAT proteins. This results in a conformational change that exposes a nuclear localization signal (NLS), promoting NFAT nuclear translocation and inducing gene expression, including various cytokines (IL-2, IL-3, IL-4, and TNFα). Members of the NFAT family have been found in many tissue types, including the heart, skeletal muscle, and brain. These transcription factors are known to be highly involved in the pathological processes of inflammation and cancer. The use of eGFP (enhanced green fluorescent protein) allows for easy assay readouts.

Application

- Screen for activators or inhibitors of NFAT signaling pathway in transduced target cells.
- Generate NFAT eGFP reporter cell pools or stable cell lines following puromycin selection.

Formulation

The lentiviruses were produced from HEK293T cells. Supplied in 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 µl x 2) of NFAT eGFP Reporter Lentivirus at ≥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 the 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.

Materials Required but Not Supplied

These materials are not supplied with this lentivirus but are necessary to follow the designed protocol. BPS Bioscience media, reagents, and luciferase assay systems 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
Thaw Medium 2	BPS Bioscience #71274
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
Anti-CD3 Agonist Antibody	BPS Bioscience #71274
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well white clear-bottom assay plate	Corning #3610

Assay Protocol

- The following protocol is a general guideline for transducing Jurkat cells. 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 target gene can be measured approximately 48-72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the target with puromycin prior to carrying out the assays.
- The assay should include “Uncoated Condition” and “Coated Condition” wells.

Day 1:

1. Harvest Jurkat cells from culture by centrifugation and resuspend the cells in fresh Thaw Medium 2.
2. Count cells.
3. Dilute Jurkat cell suspension to 2 x 10^5 cells/ ml with Thaw Medium 2.

4. Mix 500 µl of diluted Jurkat cell suspension with 400 µl of NFAT eGFP Reporter Lentivirus in a 1.5 ml Eppendorf tube.

5. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each tube to a final concentration of 8 µg/ml.

6. Gently swirl to mix.

7. Incubate for 20 minutes at Room Temperature in a tissue-culture hood.

8. Centrifuge the virus/cells mixture for 30 minutes at 800 *x g* and 32°C.

9. Remove the virus- containing medium and resuspend the cell pellet in 2 ml of fresh Thaw Medium 2.

10. Transfer the cells into one well of a 6-well plate.

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

Day 2:

1. Add 100 µl of a 10 µg/ml solution of Anti-CD3 Agonist Antibody prepared in PBS to each well of a 96-well plate. Leave a few empty wells as "Uncoated Condition".

2. Incubate overnight.

Day 3:

1. The cells are now ready for stimulation with CD3 or another assay of interest.

2. Harvest the cells and resuspend the cells in 600 µl of fresh Thaw Medium 2.

3. Wash the CD3-coated wells three times with PBS.

4. Add 100 µl of transduced Jurkat cells to the "Coated Condition" and to the "Uncoated Condition" wells.

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

Day 4:

1. The expression of eGFP can be analyzed by microscopy or flow cytometry (Ex/Em=488/510 nm), or another method of interest.

Important Notes

To generate a NFAT expressing 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, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, followed by clonal selection.

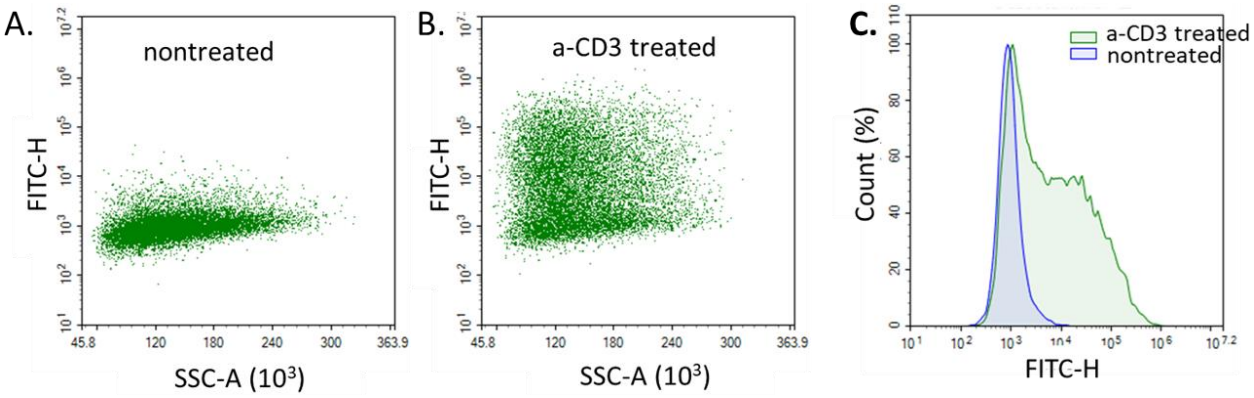

Figure 2. NFAT eGFP reporter activity stimulated by Anti-CD3 Agonist Antibody in Jurkat cells transduced with NFAT eGFP Reporter Lentivirus.
Approximately 20,000 Jurkat cells were transduced with 400,000 TU of NFAT eGFP Reporter Lentivirus. 48 hours post-transduction the medium was changed to Thaw Medium 2, and the cells were plated in pre-coated wells with Anti-CD3 Agonist Antibody for 24 hours. Non-coated wells were run in parallel as control. Expression of eGFP was observed under a fluorescence microscope (data not shown) and analyzed by flow cytometry. The y axis represents FITC intensity while the x axis represents SSC-A for transduced cells plated in non-coated (Panel A) and coated (panel B) wells. Panel C shows a comparison of transduced cells plated in non-coated (blue) and coated (green) wells, with the y axis representing % of cells and the y axis indicating FITC intensity.

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

Troubleshooting Guide
Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

References
Clipstone N.A., *et al.,* 1992 *Nature* 357(6380): 695-697.
Lyakh L., *et al.,* 1997 *Mol Cell Biol.* 17(5): 2475-2484.

Related Products

Products	*Catalog #*	*Size*
Negative Control eGFP Reporter Lentivirus	79927	500 µl x 2
NFAT Luciferase Reporter Jurkat Cell Line	60621	2 vials
NFAT Luciferase Reporter Lentivirus	79579	500 µl x 2
NFAT Reporter – HEK293 Cell Line (PKC/ Ca^{2+} Pathway)	79298	2 vials
NFAT Reporter (Luciferase) – THP1 Cell Line	78320	2 vials
NFAT Luciferase- RFP Reporter Lentivirus	78382	500 µl x 2

Version 040924