6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

NFAT Luciferase Reporter Lentivirus

Catalog #: 79579

Product Description
The NFAT 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 driven by the NFAT response element located upstream of the minimal TATA promoter (Figure 1). After transduction, activation of the NFAT signaling pathway in the target cells can be monitored by measuring the luciferase activity.

Application
- Screen for activators or inhibitors of NFAT signaling pathway in transduced target cells
- Generation of NFAT Luciferase Reporter stable cell line

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

Titer
Two vials (500 µl x 2) of NFAT 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 third 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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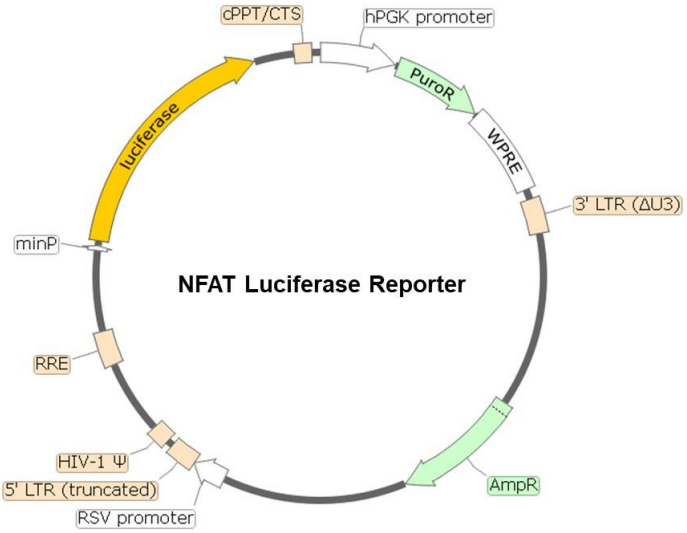

Figure 1. Schematic of the lenti-vector used to generate the NFAT luciferase reporter lentivirus

Materials Required but Not Supplied

- Jurkat cells (ATCC # TIB-152)
- Anti-CD3 agonist antibody (BPS Bioscience, #71274)
- Jurkat growth medium (Thaw Medium 2, BPS Bioscience, #60184)
- 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 Jurkat cells using NFAT 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. Precoat the 96 well plate with anti-CD3 antibody in PBS overnight. Leave a few noncoated wells to serve as negative controls. Rinse all wells 3x with PBS.

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2. Harvest the Jurkat cells by centrifugation and resuspend the cells in fresh Thaw Medium 2. Dilute the cells to 2 x 10^5 /ml in growth medium. Mix 500 µl of the Jurkat cells and 400 µl of NFAT luciferase reporter lentivirus in a 1.5-ml Eppendorf tube. Add polybrene to a final concentration of 8 µg/ml. Gently mix and incubate the virus with the Jurkat cells for 20 min at room temperature in the tissue culture hood.

3. 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 Thaw Medium 2. Transfer the cells into one well in a 6-well plate. Incubate the plate at 37°C with 5% CO_2 for 48-66 hours. The transduced Jurkat cells are ready for assay development.

4. Harvest the cells and resuspend the cells into 600 µl of fresh Thaw Medium 2. Add 100 µl of the cells to each well of the CD3 antibody-coated 96-well plate.

5. Incubate at 37°C with 5% CO_2 for 5-6 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 NFAT 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) Reporter 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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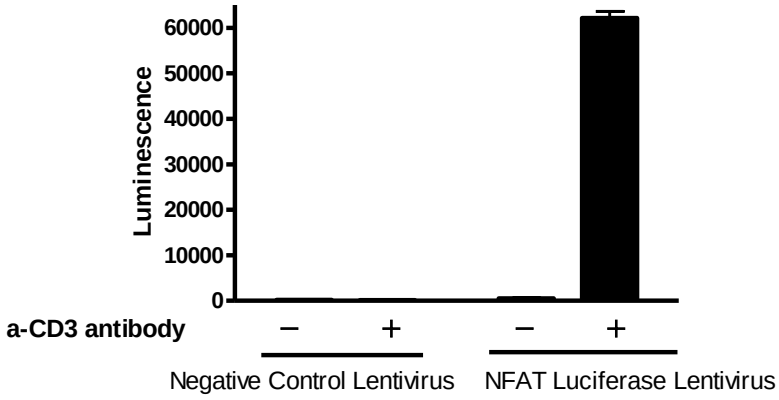

Figure 2. NFAT luciferase reporter activity stimulated by anti-CD3 agonist antibody in Jurkat cells. Appropriate 20,000 Jurkat cells were transduced with 300,000 TU NFAT luciferase reporter lentivirus. After 66 hours of transduction, medium was changed to Thaw medium 2. Cells were stimulated with anti-CD3 agonist antibody (precoated on a 96-well plate) for ~6 hours. The noncoated wells and the negative control lentivirus were performed in parallel as controls. The results are shown as the raw luminescence reading.

Related Products

Product	**Cat. #**	**Size**
NF-κB Luciferase Reporter Lentivirus	79564	500 µl x2
CRE/CREB Luciferase Reporter Lentivirus	79580	500 µl x2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x2
TCF/LEF Luciferase Reporter Lentivirus	79787	500 µl x2
Reporter 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
LentiLight GFP Lentivirus	79703	1 ml x 2
NFAT Reporter – Hek293 Cell Line (PKC/ Ca2+ Pathway)	79298	2 vials
NFAT Reporter (Luc) – Jurkat Recombinant Cell Line	60621	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

References

Clipstone NA, Crabtree GR. *Nature*. 1992 Jun 25;**357(6380):**695-7.

Lyakh, L., *et al. Mol Cell Biol*. 1997 May;**17(5):**2475-84.