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# Produktinformation

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- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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# Data Sheet

## *NFAT eGFP Reporter Lentivirus*

### *Catalog #: 79922*

**Product Description**
The NFAT eGFP 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 an enhanced GFP 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 examining eGFP expression.

**Application**
- Screen for activators or inhibitors of NFAT signaling pathway in transduced target cells
- Generation of NFAT eGFP 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 eGFP 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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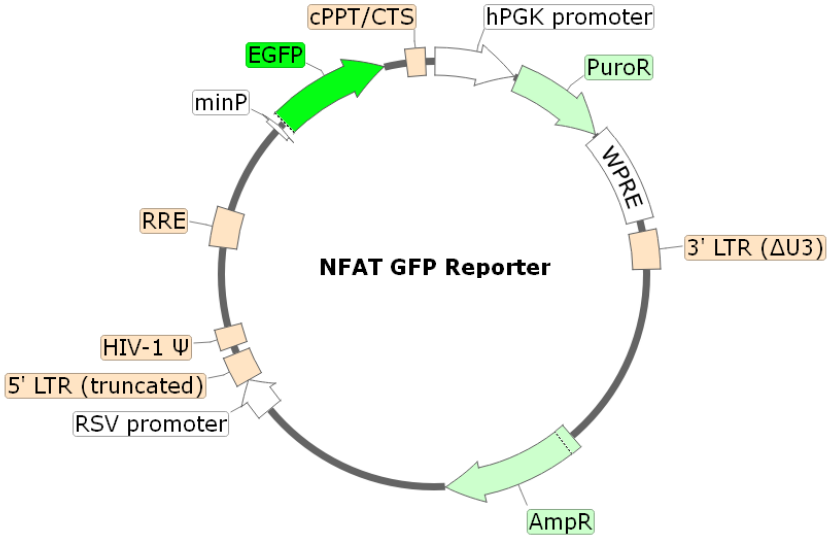


**Figure 1. Schematic of the lenti-vector used to generate the NFAT eGFP reporter lentivirus**

**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)

**Assay protocol**

The following protocol is a general guideline for transducing Jurkat cells using NFAT 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. Precoat the 96 well plate with 100 µl of anti-CD3 antibody at 10 µg/ml in PBS overnight. Leave a few noncoated wells to serve as negative controls. Rinse all wells 3x with PBS.

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 Thaw Medium 2. Mix 500 µl of the Jurkat cells and 400 µl of NFAT GFP 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 Jurkat cells for 20 minutes 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. The same amount of the cells was added to the noncoated wells to serve as a control.

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

6. The expression of eGFP can be analyzed by microscopy or flow cytometry (Ex/Em = 488/510 nm).

Important Notes:

To generate the NFAT 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.

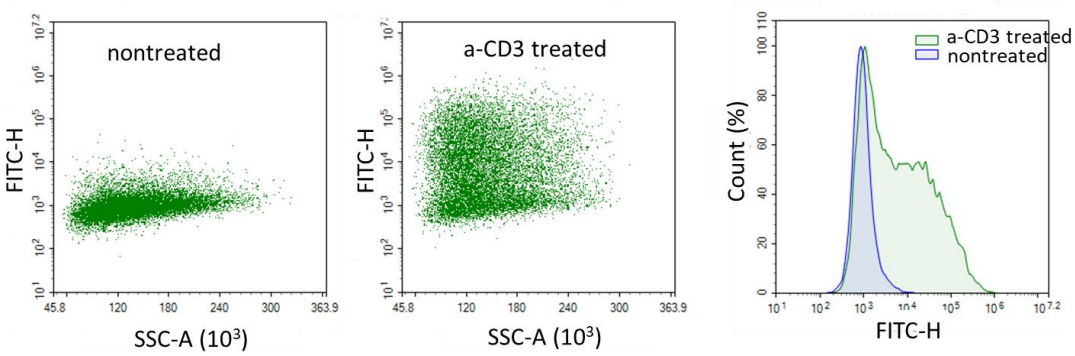


**Figure 2. NFAT eGFP reporter activity stimulated by anti-CD3 agonist antibody in Jurkat cells.** Appropriate 20,000 Jurkat cells were transduced with 400,000 TU NFAT eGFP reporter lentivirus. After 48 hours of transduction, medium was changed to Thaw medium 2. Cells were stimulated with anti-CD3 agonist antibody (precoated on a 96-well plate) for ~24 hours. The noncoated wells were performed in parallel as controls. The eGFP expression was analyzed by fluorescence microscopy (data not shown) and flow cytometry.

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**Related Products**

| Product | Cat. # | Size |
|---|---|---|
| NFκB 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 |
| STAT5 Luciferase Reporter Lentivirus | 79745 | 500 µl x2 |
| TCF/LEF Luciferase Reporter Lentivirus | 79787 | 500 µl x2 |
| ISRE Luciferase Reporter Lentivirus | 79824 | 500 µl x2 |
| IL-2 Promoter Luciferase Reporter Lentivirus | 79825 | 500 µl x2 |
| IL-8 Promoter Luciferase Reporter Lentivirus | 79827 | 500 µl x2 |
| AP-1 Luciferase Reporter Lentivirus | 79823 | 500 µl x2 |
| SBE Luciferase Reporter Lentivirus | 79806 | 500 µl x2 |
| TEAD Luciferase Reporter Lentivirus | 79833 | 500 µl x2 |
| ARE Luciferase Reporter Lentivirus | 79869 | 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 |
| FcGRIIIA Lentivirus | 79876 | 500 µl x2 |
| FcGRIIB Lentivirus | 79877 | 500 µl x2 |
| FcER1G Lentivirus | 79878 | 500 µl x2 |
| Expression negative Control Lentivirus | 79902 | 500 µl x2 |
| Secreted Gaussia Lentivirus | 79892 | 500 µl x2 |
| Non-Secreted Gaussia Luciferase Lentivirus | 79893 | 500 µl x2 |
| TCR Activator Lentivirus | 79894 | 500 µl x2 |