



## Reporting Lentivirus for detection of NFAT Signaling Pathway Activity

Cat#	Product Name	Amounts
<a href="#">LVP1711</a>	Luc (Puro) NFAT Pathway Lentivirus	200ul x (1 x 10 <sup>8</sup> IFU/mL)
<a href="#">LVP1712</a>	GFP (Puro) NFAT Pathway Lentivirus	
<a href="#">LVP1713</a>	Luc (GFP) NFAT Pathway Lentivirus	

**Storage:** Kept at -80 °C freezer, avoid repeat freeze/thaw cycles. Stable for 6 months after the receiving.

### 1. Product Description:

The lentiviral system is a gene delivery tool that uses lentivectors for gene expression or knockdown. GenTarget's lentivector system is based on Human Immunodeficiency Virus-1 (HIV) plasmids for these purposes. Lentivectors are used to generate lentiviral particles (lentivirus), which can be transduced into nearly all types of mammalian cells, including stem cells, primary cells, and non-dividing cells, both in vivo and in vitro. Lentiviral particles stably integrate into the genome of transduced cells, ensuring long-term expression, making them an excellent gene transfer agent.

NFAT (Nuclear Factor of Activated T-cells) proteins are transcription factors that play essential roles in T-cell activation, development, and function, as well as in other cell types. They are regulated by their phosphorylation status, which affects their localization and activity. NFAT signaling pathway is activated by an increase in intracellular calcium levels, which dephosphorylates NFAT proteins. Dephosphorylated NFAT translocates from the cytoplasm to the nucleus, binds to specific DNA sequence, the transcriptional response element (TRE) in the promoter, leading to the activation of its target genes [\[1\]](#).

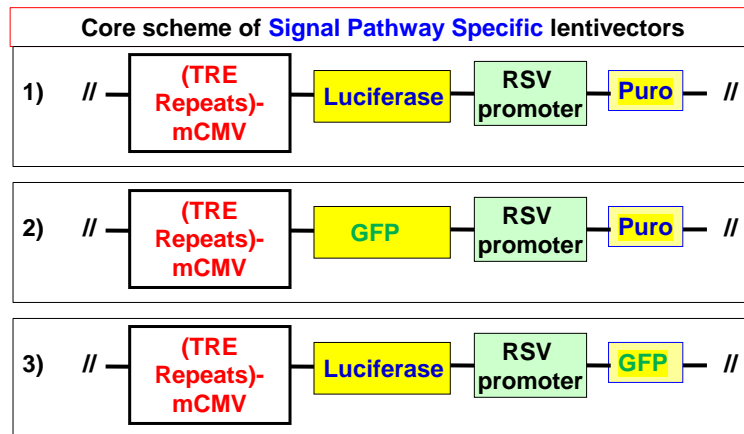
NFAT signaling pathway is a vital regulator of immune function, development, and cellular responses to stimuli. Its precise regulation is crucial for maintaining normal physiological functions, and its dysregulation can lead to various pathological conditions.

### 2. NFAT pathway Lentivectors:

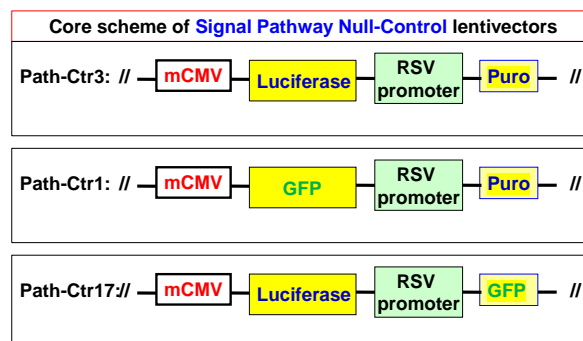


GenTarget developed a set of reporting lentivirus products for monitoring or detecting the NFAT pathway's activity in any of your desired cell types. Those reporting lentivirus has a **luminescent report (Firefly-Luciferase)** or a **fluorescent report (GFP)**, under the NFAT responsive promoter of minimal CMV promoter (mCMV) containing multiple pathway specific tandem repeats ( 5'- GGAGGAAAAAC-TGTTTCATACAGAAGGCGT-TGGAAACTC 3') [2] as the transcriptional response element (TRE).

Those reporting lentivirus also constitutive express the **Puromycin** antibiotic selection marker or **GFP** fluorescent selection marker under a constitutive RSV promoter, which provides the selection of the transduced cells (to generate pathway specific sensor cell lines), via puromycin killing or GFP positive cell sorting. See lentivector's core scheme below.



A set of corresponding **pathway Null-Control lentivirus** were also generated. Those control use the minimal promoter that does not contain any signal pathway's TRE sequences, and will not response to pathway's induction or treatment. The pathway control lentivirus are used to set the signal reference for the specificity of pathway treatment. See the scheme below for the pathway control lentivectors.





### 3. Premade NFAT signal reporting lentivirus:

GenTarget produced the replication incompetent lentivirus using 3<sup>rd</sup>-generation lentiviral packaging system. Lentiviruses are HIV-based, pseudotyped with VSVG envelope protein, produced in 293T cells. All particles were tested to be free of bacterial and mycoplasma contamination. Virus titers were tested lot by lot.

The ready-to-use lentivirus expresses a report: **firefly Luciferases (Luc)**, or **GFP fluorescent report** under NFAT pathway responsive promoter. The NFAT pathway is activated primarily by signals that increase intracellular calcium levels. Upon activation (dephosphorylates), the NFAT proteins bind to the TRE in report's promoter region, leading to increased report expression, the Luc or GFP.

Each lentivirus product also contains a constitutively expressed selectable marker: Puromycin (**Puro**), or **GFP** fluorescent.

The lentivirus is ready and easy to use, simply add 50ul into one well of your cell culture in 24-well plate, and select or sort the positive transduced cells at 2-3 days post virus transduction (for screening cell line generation). Or simply go for signal pathway specific induction assays without the selection (for transient assay). The readout can be easily monitored by luciferase signal or via the GFP fluorescent signal depending on the product.

The premade reporter lentivirus provides an easier, sensitive and quantitative tool to monitor activity of the signaling pathways, or screen the components that affect the pathway specific transcriptional factors. It allows to generate your reporting cell line in your desired cell type, for the study or screening of pathway specific chemicals / drug / protein in the cell-based assay.

### 4. Application for Pathway Reporting Lentivirus:

- 1) Create signal pathway specific cell lines for a High-throughput, live cell-based assays for signal transduction tests;
- 2) Identify or validate the signaling pathway specific drugs (drug discovery and validation);
- 3) Analyze the pathway-specific responses to proteins, peptides, or hormones;
- 4) Analyze the pathway-specific responses to gene activation, over-expression, knockdown, knockout, or mutagenesis;



- 5) Screen for pathway-specific stimulus or for the transcriptional activators that response to specific pathway's TRE elements;
  - 6) makes it easy to measure the transcriptional and post-transcription regulation in response to signal pathway stimulus.
- For more details about premade particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf).

## 5. Transduction Protocols (How to use the lentivirus):

**Simple Protocol:** Pre-made lentivirus is provided ready to use, so it can be simply added into your cell culture; the amount of virus to add depends on cell type. For quick transduction, add 50  $\mu$ l of virus into each well of 24-well-plate where cell density is 50% to 75% (no need to worry about MOI unless you want to control the inserted copy numbers of expression cassette). After 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy if applicable. For stable cell line generation, pass cells into selection medium containing Puromycin, or perform GFP fluorescence cell sorting.

### Day 0:

Seed cells in complete medium at the appropriate density and incubate overnight.

**Note:** at the time of transduction, cells should be 50%-75% confluent. For example, seed HeLa cells at  $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$  in a well of a 24-well plate.

### Day 1:

- 1) Thaw the lentivirus products at room temperature and add the 50ul of lentivirus into one well. (Or add the amount to obtain the desired MOI. If desirable, please set up the controls by using Path-control lentivirus.
- 2) Return cells to 37°C, CO<sub>2</sub> incubator.

**Note:** If you do not use up all virus at one time, you may re-freeze the virus at -80 °C for future use; virus titer will decrease by ~10% for each freeze/thaw cycle. Try to avoid freezing and thawing.

### Day 3:

- 1) At ~72hr after transduction, if applicable, check the transduction rate by GFP positive cell counts under fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS).

**Note: Filter wavelength settings:**

GFP filter: ~Ex450-490 ~Em525;



- 2) Enrich the positive transduced cells by sorting the GFP positive cells (if applicable), or by Puromycin killing selection. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line

### **Day 3 +:**

- 1) Grow up the positive transduced cells. The selected stable cells will be used for signal pathway assay with desired treatment. (Note: the selected Pathway Control cells serve as the non-pathway specific signal background).
- 2) Treat the cells with signal pathway inducers or inhibitors, and analyze the pathway reporter expression (GFP Fluorescent readout, or luciferase assay depend upon the products).

## **6. Signal pathway assay recommendations:**

- 1) Treatment: the reporter's inducible expression or inhibition is dose and time dependent upon induction or treatment. You may need to optimize the best treatment amount and the time point.
- 2) Controls:
  - Pathway Null response Controls (CAT#: [Path-Ctr3](#); [Path-Ctr1](#) to [Path-Ctr17](#)): Gentarget's Pathway control lentivirus contains the minimal promoter in the same lentivector backbones as for the pathway lentivirus. The minimal promoter, demonstrated weak promoter strength in most cell types, drives the report expression which services as the signal control for pathway non-specific response.
  - Positive response controls: If applicable, apply the characterized pathway stimulus as the pathway positive induction controls, such as treated with known inducers (or inhibitors), proteins, peptide or compounds.
- 3) Make triplicates for each condition for assay reproducibility.
- 4) Assay cell number: you may need to carry out a cell titration to determine the optimal cell number for the signal reporter assay.

## **7. Safety Precaution:**

Gentarget lentiviral particles adapts must advanced lentiviral safety features (using the third generation vectors with self-inactivation SIN-3UTR), and the



premade lentivirus is replication incompetent. However, please use extra caution when using lentiviral particles. Use the lentiviral particles in Bio-safety II cabinet. Wear glove all the time at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

## 8. References:

- 1) *Revisiting the Concept of Targeting NFAT to Control T Cell Immunity and Autoimmune Diseases; Front. Immunol., 26 November 2018*
- 2) *Transcriptional Regulation of TMP21 by NFAT; Molecular Neurodegeneration volume 6, Article number: 21 (2011)*

## 9. Warranty:

**This product is for research use only.** It is warranted to meet its quality as described when used in accordance with its instructions. GenTarget disclaims any implied warranty of this product for particular application. In no event shall GenTarget be liable for any incidental or consequential damages in connection with the products. GenTarget's sole remedy for breach of this warranty should be, at GenTarget's option, to replace the products.

10. **Attachment:** GenTarget's pre-made lentivirus product categories.

<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">Pathway Reporter</a>	Repoter Lentivirus for all kinds of pathway screening assays
<a href="#">Cell Immortalization</a>	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.
<a href="#">ImmunoOncology Research</a>	Lentivirus products for immuno therapy research: CAR and TCR; Assay Cell Lines for T-cell targeted killing assay and other cell-based assays; over-expression lentivirus products for the immune response targets; Cell surface antigens (CDs); immune checkpoint / Receptors; CRISPR gene Repair and knock-IN lentivirus; CRISPR knockout lentivirus;
<a href="#">CAR-T, TCR Lentivirus</a>	<b>CARs</b> Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; <b>TCRs</b> : MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;
<a href="#">CRISPR Gene Editing</a>	Preamde lentivirus express humanized wild-type <b>Cas9</b> endonuclease, the <b>dCas9</b> , gRNAs, <b>CRISPR</b> gene editing



<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
	research
<a href="#">Epigenomic: CRISPRi and CRISPRa</a>	" <b>dCas9-Protein</b> " fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
<a href="#">Cell-Specific Reporter</a>	a set of reporter lentiviruses to express a luminescence or fluorescent reporter (firefly Luciferase, Renilla luciferase, RFP or GFP fluorescent marker) under a tissue specific promoter
<a href="#">Infectious Antigens</a>	Lentivirus that express all kinds of infectious antigens with C-term 6His-tag.
<a href="#">Virus Like Particles (VLP)</a>	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
<a href="#">Non-integrating LV</a>	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
<a href="#">shRNA Knockdown</a>	Knockdown verified and customized shRNA lentivirus for target knockdown,
<a href="#">microRNA lentivirus</a>	Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human and mouse miRNA.
<a href="#">Anti-miRNA lentivirus</a>	Pre-made lentivirus expression a specific anti-miRNA cassette.
<a href="#">Human and mouse ORFs</a>	Premade lentivirus expressin a <b>human, mouse or rat</b> gene with RFP-Blastididin fusion dual markers.
<a href="#">Luciferase expression</a>	Premade lentivirus for all kinds of luciferase protein expression: <b>firefly and Renilla, Red-Luc and more</b> , with different antibiotic selection markers.
<a href="#">Fluorescent Markers</a>	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.
<a href="#">Luminescent Imaging</a>	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
<a href="#">Sub-cellular Imaging</a>	Lentivirus contain a well-defined organelle targeting signal fused to a fluorescent protein, great tools for



<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
	live-cell imaging and for dynamic investigation of sub-cellular signal pathways.
<a href="#">Cytoskeleton Imaging</a>	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
<a href="#">Unstable GFP</a>	Lentivirus express the the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection
<a href="#">near-infrared RFP</a>	The near-infrared Red fluorescent (niRFP) expression Lentiviurs provides the whole-body images with better contrast and brighter images
<a href="#">Fluorescent-ORF fusion</a>	Pre-made lentivirus expression a " <b>GFP/RFP/CFP-ORF</b> " fusion target.
<a href="#">CRE recombinase</a>	Premade lentivirus for expressing <b>nuclear permeant CRE</b> recombinase with different flurescent and antibiotic markers.
<a href="#">CRE, Flp ColorSwitch</a>	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or "FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the CRE or Flp recombination event in vivo.
<a href="#">SEAP Reporter</a>	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
<a href="#">TetR Repressor</a>	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
<a href="#">rtTA Expression</a>	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF inducible system.
<a href="#">iPS factors</a>	Premde lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FLF4</b> ) factors with different fluorescent and antibiotic markers
<a href="#">LacZ expression</a>	Express different full length <b>β-galactosidase (lacZ)</b> with different selection markers
<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentiviruses treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme</a>	Ready-to-use lentivirus, expressing a specific enzymes





<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">expression</a>	with different selection markers.
<a href="#">Ultra titer lentivirus</a>	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.