



Pre-made Reporter Lentivirus for NFkB Signal Pathway

Cat#	Product Name	Amounts
LVP965-P or: LVP965-P-PBS	NFKB- GFP (Puro) Lentivirus	200ul, $\sim 1 \times 10^7$ IFU/mL in DMEM medium with 10x polybrene. Or 200ul, $\sim 1 \times 10^8$ IFU/mL in PBS solution
LVP966-P or: LVP966-P-PBS	NFKB- RFP (Puro) Lentivirus	
LVP967-P or: LVP967-P-PBS	NFKB- Luc (Puro) Lentivirus	
LVP968-P or: LVP968-P-PBS	NFKB- Rluc (Puro) Lentivirus	
LVP1292 or: LVP1292-PBS	NFKB- SEAP (Puro) Lentivirus	
LVP965-B or: LVP965-B-PBS	NFKB- GFP (Bsd) Lentivirus	
LVP966-B or: LVP966-B-PBS	NFKB- RFP (Bsd) Lentivirus	
LVP967-B or: LVP967-B-PBS	NFKB- Luc (Bsd) Lentivirus	
LVP968-B or: LVP968-B-PBS	NFKB- Rluc (Bsd) Lentivirus	
LVP1293 or: LVP1293-PBS	NFKB- SEAP (Bsd) Lentivirus	
LVP965-N or: LVP965-N-PBS	NFKB- GFP (Neo) Lentivirus	
LVP966-N or: LVP966-N-PBS	NFKB- RFP (Neo) Lentivirus	
LVP967-N or: LVP967-N-PBS	NFKB- Luc (Neo) Lentivirus	
LVP968-N or: LVP968-N-PBS	NFKB- Rluc (Neo) Lentivirus	
LVP1294 or: LVP1294-PBS	NFKB- SEAP (Neo) Lentivirus	
LVP965-R or: LVP965-R-PBS	NFKB- GFP (RFP) Lentivirus	
LVP967-R or: LVP967-R-PBS	NFKB- Luc (RFP) Lentivirus	
LVP968-R or: LVP968-R-PBS	NFKB- Rluc (RFP) Lentivirus	



LVP1295 or: LVP1295-PBS	NFKB- SEAP (RFP) Lentivirus	
LVP966-G or: LVP966-G-PBS	NFKB- RFP (GFP) Lentivirus	
LVP967-G or: LVP967-G-PBS	NFKB- Luc (GFP) Lentivirus	
LVP968-G or: LVP968-G-PBS	NFKB- Rluc (GFP) Lentivirus	

Storage: <-70 °C, avoid repeat freeze/thaw cycles. Stable for >6 months.

Product Description:

Lentiviral system is a gene delivery tool using lentivectors for gene expression or knockdown. GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into almost all kinds of mammalian cells, including stem cells, primary cells, and non-dividing cells both *in vivo* and *in vitro*. Lentiviral Particles stably integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.

NFkB signaling pathways:

NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls transcription of DNA, cytokine production and cell survival. NF-κB plays a key role in inflammation, immune response, cell proliferation and protection against apoptosis, involves in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens. Incorrect regulation of NF-κB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development. NF-κB has also been implicated in processes of synaptic plasticity and memory.

There are five proteins in NFkB family including RelA, RelB, Rel, NF-κB1 (p105 / p50) and NF-κB2 (p100 / p52). A variety of extracellular signals can activate the enzyme IκB kinase (IKK). IKK, in turn, phosphorylates the IκBα protein, which results in ubiquitination, dissociation of IκBα from NF-κB, and eventual degradation of IκBα by the proteasome. The activated NF-κB is then translocated into the nucleus where it binds to specific sequences of DNA called transcriptional response element (**NFkB-TRE**). The DNA/NF-κB complex then recruits other proteins such as coactivators and RNA



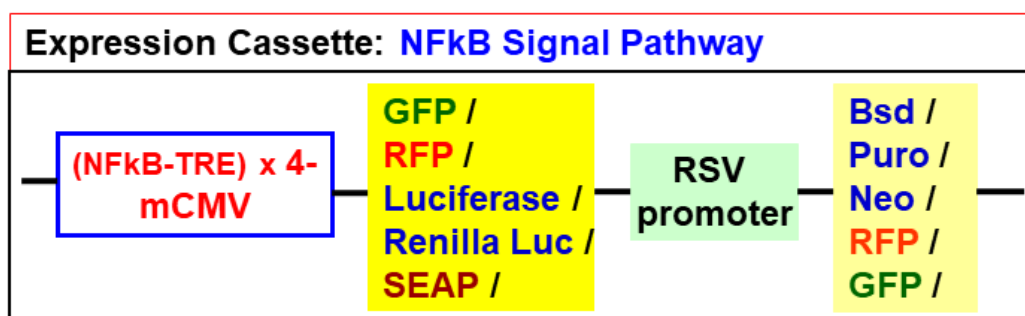
polymerase, which transcribe downstream DNA into mRNA, which, in turn, is translated into protein, which results in a change of cell function.

Product Principle:

GenTarget developed a set of reporting lentivirus to monitor NFκB-regulated signaling pathway. Those reporting lentivirus has a **luminescent report (Luciferase, Renilla Luc)** or a **fluorescent report (GFP, RFP)** or a secreted **SEAP** report, under a minimal CMV promoter (mCMV) that embedded with optimized tandem repeats of NFκB transcriptional response element (NFκB-TRE) sequence motif (5'- **GGGACTTCC**). After cells are treated by pro-inflammatory cytokines or stimulants of lymphokine receptors, the NF-κB transcription factors will bind to NFκB-TRE, which activates the minimal promoter, resulted in the downstream reporter's expression. This pathway lentivirus can be verified by recombinant human tumor necrosis factor alpha (hTNFα) protein.

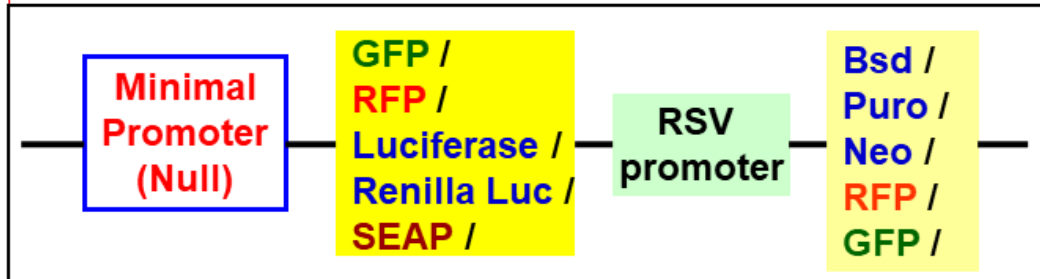
The luciferase signal can be measured via Luciferase assay. The fluorescent reporter can be detected via its fluorescent signal. The SEAP secreted into cell culture supernatant, which allows to determine reporter activity without disturbing the cells, does not require the preparation of cell lysates and can be used for kinetic studies.

Those reporting lentivirus also contains a constitutively expressed fluorescent selection marker or an antibiotic selection marker under the RSV promoter, which makes it easier to select the stably infected signal reporting cells (to generate pathway specific sensor cell lines), or provides internal reference for virus transduction efficiency when a fluorescent marker is under the RSV promoter. A set of control lentivirus use the same lentivector backbones except the minimal promoter does not contain any signal pathway's TRE sequences. The control lentivirus are used to set the reference for specificity of pathway signal response upon treatment. See the scheme below for lentivector's core expression cassette.





Lentivector: Pathway Null Controls (CAT#: Path-Ctr)



The premade, ready-to-use reporter lentivirus provides a much easier tool to monitor the activity of NFkB signaling pathways in virtually any mammalian cell type. It also allows to generate your own reporting cell line in your desired cell type for study or screen of pathway specific gene-knockdown, over-expression, or chemical / drug/protein treatment in the cell based assay.

Lentivirus are HIV-based, pseudotyped with VSVG envelope protein, produced in 293T cells. All particles were tested to be free bacterial and mycoplasma contamination. Virus titers were tested lot by lot.

Key Application for Pathway Signaling Lentivirus:

1. Create signal pathway specific cell lines which can provide 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.

Product Formats:

The pre-made lentivirus provided in two formats:

1. Packaged in 10% of FBS in DMEM containing 10% FBS and 60ug/ml of polybrene (10x);
2. Particles were concentrated and buffer exchanged in PBS without any human or animal origin components. The virus in PBS are used for any cell



types that requires non-serum in the culture medium, or best for the hard-to-infect cell types.

The lentivirus are 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 sensor cell line assay). Or simply go for Estrogen receptor signal induction without the selection (for transient assay). The readout can be easily monitored by luciferase assay or via the Fluorescent microscope or readers depending on product report type.

For more details about premade particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf).

Transduction Protocols:

Note: 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 µl of virus into each well of 24-well-plate where cell density is 50% to 75%. After 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy (when applicable). For stable cell line generation, pass cells into medium containing antibiotic for selection, or perform 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:

- Remove the culture medium and add 0.5ml fresh, warm, complete medium.
- Thaw the pre-made lentiviral stock at room temperature and add the appropriate amount of virus stock to obtain the desired MOI.
- Return cells to 37°C, CO₂ incubator.

Note: Try to avoid freezing and thawing. 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.

Day 3:

At ~72hr after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by



flow cytometry (FACS or Guava). You can now treat the cell for signal pathway assay.

Day 3 + (optional):

Sort transduced cells by FACS, and select for antibiotic resistance. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line (refer to the pertinent literature on generation of stable cell lines). The selected stable cells will be used for signal pathway assay with desired treatment.

Next: Treat the cell with signal pathway inducer, and analyze the pathway reporter expression (Fluorescent readout or luciferase assay).

Signal pathway assay recommendations:

1. **Treatment:** the reporter's inducible expression is dose and time dependent upon induction or treatment. You may need to optimize the best treatment amount and the time point.

2. Controls:

- Virus transduction controls: Gentarget's signal report lentivirus contains a non-inducible fluorescent marker as the internal normalization control (only applicable to the lentivirus containing a fluorescent marker under Rsv promoter). The embed internal control fluorescent signal also monitors the lentivirus transduction efficiency in assay cell types. When the internal control is not available, you can mix a regular luciferase or fluorescent marker lentivirus under a constitutive promoter (see Gentarget's [luciferase lentivirus](#) and [fluorescent lentivirus](#) products).
- No-response controls: if desired, you also can use the [Pathway-control lentivirus](#) that made in the same lentivector backbone but without any TRE in its minimal promoter. (Note: the minimal CMV promoter has no or little activity in most cell types). This control virus serves for the specificity of any treatment or as for the establishment of the basal signal profile.
- Positive controls: If applicable, apply the characterized pathway stimulus as the pathway positive induction controls, such as treated with known inducer, 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.

Safety Precaution:

Gentarget lentiviral particles adapt 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.

References:

- Science. 2001; 293 (5534): 1495–9.
- Science. 2004; 306 (5696): 704–8.
- Nature Reviews. Immunology. 2002; 2 (10): 725–34.

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.

Note: Filter wavelength settings:

GFP filter: ~Ex450-490 ~Em525; **RFP filter:** ~Ex545 ~Em620;

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

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Repoter Lentivirus for all kinds of pathway screening assays
Cell Immortalization	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.



Product Category	Product Description (please click into each category's page)
ImmunoOncology Research	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;
CAR-T, TCR Lentivirus	CARs Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; TCRs : MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRaV3-F2A-TRβV5-6;
CRISPR Gene Editing	Preamde lentivirus express humanized wild-type Cas9 endonuclease, the dCas9 , gRNAs, CRISPR gene editing research
Epigenomic: CRISPRi and CRISPRa	" dCas9-Protein " fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
Cell-Specific Reporter	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
Infectious Antigens	Lentivirus that express all kinds of infectious antigens with C-term 6His-tag.
Virus Like Particles (VLP)	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
Non-integrating LV	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
shRNA Knockdown	Knockdown verified and customized shRNA lentivirus for target knockdown,
microRNA lentivirus	Premade lentivirus expression human or mouse precursor miRNA . And anti-miRNA lentivector and virus for human and mouse miRNA.
Anti-miRNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Human and mouse ORFs	Premade lentivirus expressin a human, mouse or rat gene with RFP-Blastididin fusion dual markers.



Product Category	Product Description (please click into each category's page)
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression: firefly and Renilla, Red-Luc and more , with different antibiotic selection markers.
Fluorescent Markers	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.
Luminescent Imaging	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
Sub-cellular Imaging	Lentivirus contain a well-defined organelle targeting signal fused to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways.
Cytoskeleton Imaging	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
Unstable GFP	Lentivirus express the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection
near-infrared RFP	The near-infrared Red fluorescent (niRFP) expression Lentiviruses provides the whole-body images with better contrast and brighter images
Fluorescent-ORF fusion	Pre-made lentivirus expression a " GFP/RFP/CFP-ORF " fusion target.
CRE recombinase	Premade lentivirus for expressing nuclear permeant CRE recombinase with different fluorescent and antibiotic markers.
CRE, Flp ColorSwitch	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.
SEAP Reporter	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
TetR Repressor	Premade lentivirus expressing TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF



Product Category	Product Description (please click into each category's page)
	inducible system.
iPS factors	Premade lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FLK4) factors with different fluorescent and antibiotic markers
LacZ expression	Express different full length β-galactosidase (lacZ) with different selection markers
Negative control lentiviruses	Premade negative control lentivirus with different markers : serves as the negative control of lentivirus treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.
Ultra titer lentivirus	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.