GenTarget Inc
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Pre-made Reporting Lentivirus for Wnt Signaling Pathway Activity

Cat#	Product Name	Amounts
LVP808-P or LVP808-P-PBS	Wnt Tcf-**GFP** (Puro) Lentiviral particles	200ul, ~1 x 10^7 IFU/mL in DMEM containing 10% FBS Or 200ul, ~1 x 10^8 IFU/mL in PBS solution
LVP809-P or LVP809-P-PBS	Wnt Tcf-**RFP** (Puro) Lentiviral particles	
LVP810-P or LVP010-P-PBS	Wnt Tcf-**Luc** (Puro) Lentiviral particles	
LVP811-P or LVP811-P-PBS	Wnt Tcf-**Rluc** (Puro) Lentiviral particles	
LVP808-B or LVP808-B-PBS	Wnt Tcf- **GFP** (Bsd) Lentiviral particles	
LVP809-B or LVP809-B-PBS	Wnt Tcf- **RFP** (Bsd) Lentiviral particles	
LVP810-B or LVP810-B-PBS	Wnt Tcf- **Luc** (Bsd) Lentiviral particles	
LVP811-B or LVP811-B-PBS	Wnt Tcf- **Rluc** (Bsd) Lentiviral particles	
LVP808-N or LVP808-N-PBS	Wnt Tcf- **GFP** (Neo) Lentiviral particles	
LVP809-N or LVP809-N-PBS	Wnt Tcf- **RFP** (Neo) Lentiviral particles	
LVP810-N or LVP810-N-PBS	Wnt Tcf- **Luc** (Neo) Lentiviral particles	
LVP811-N or LVP811-N-PBS	Wnt Tcf- **Rluc** (Neo) Lentiviral particles	
LVP808-R or LVP808-R-PBS	Wnt Tcf- **GFP** (RFP) Lentiviral particles	
LVP810-R or LVP810-R-PBS	Wnt Tcf- **Luc** (RFP) Lentiviral particles	
LVP811-R or LVP811-R-PBS	Wnt Tcf- **Rluc** (RFP) Lentiviral particles	
LVP809-G or LVP809-G-PBS	Wnt Tcf- **RFP** (GFP) Lentiviral particles	
LVP810-G or LVP810-G-PBS	Wnt Tcf- **Luc** (GFP) Lentiviral particles	
LVP811-G or LVP811-G-PBS	Wnt Tcf- **Rluc** (GFP) Lentiviral particles	

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

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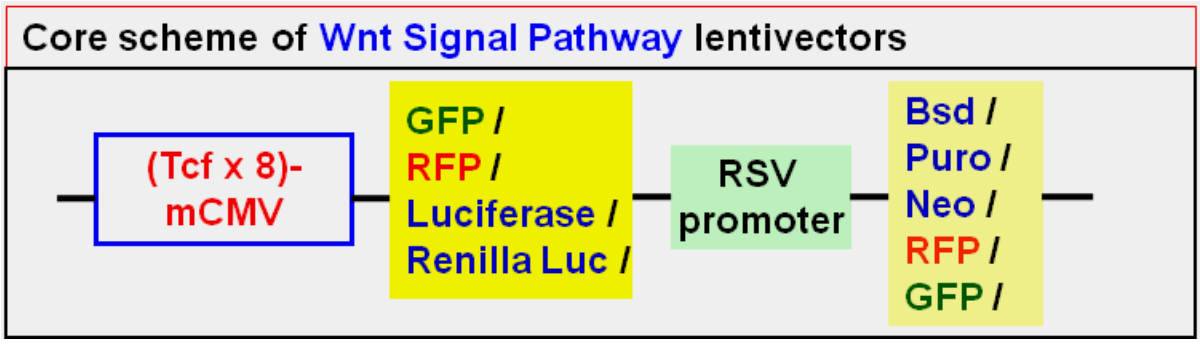
(lentivirus) that can be transduced into almost all kinds of mammalian cells, including stem cells, primary cells, and non-dividing cells both *in vivo* and *in vitro*. Lentiviral Particles stably integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.

The **Wnt signaling pathways** are a group of signal transduction pathways made of proteins that pass signals from outside of a cell through cell surface receptors to the inside of the cell. Wnt signaling pathways are activated by the binding of a Wnt-protein (a large family of secreted glycoproteins, such as wnt3A, Wnt1, and so on) to a family receptor. The Wnt signaling pathway plays important roles in cell cell proliferation, differentiation and survival and many other cell developing aspects.

GenTarget developed a set of reporting lentivirus for monitoring or manipulating the Wnt pathway's activity in any of your desired cell types. Those reporting lentivirus has a luminescent report or a fluorescent report under the Wnt-responsive promoter the minimal CMV promoter (mCMV) containing 8xTcf tandem repeats as the transcriptional response element (TRE). The Tcf repress the report expression in the absence of the Wnt signal/inducer. Once the inducer (like Wnt3A protein) is present, it binds to promoter's TRE, initialing the expression of the downstream luminescent or fluorescent report, which can be easily readout via luciferase assay or by fluorescent microscope.



Those reporting lentivirus also constitutive express an antibiotic selection marker or a different fluorescent selection marker under a constitutive RSV promoter, which provides the selection for the stable signal reporting cells (to generate pathway specific sensor cell lines), or this constitutively expressed fluorescent marker (when applicable) can be serve as the internal normalization control. See lentivector's core scheme above.

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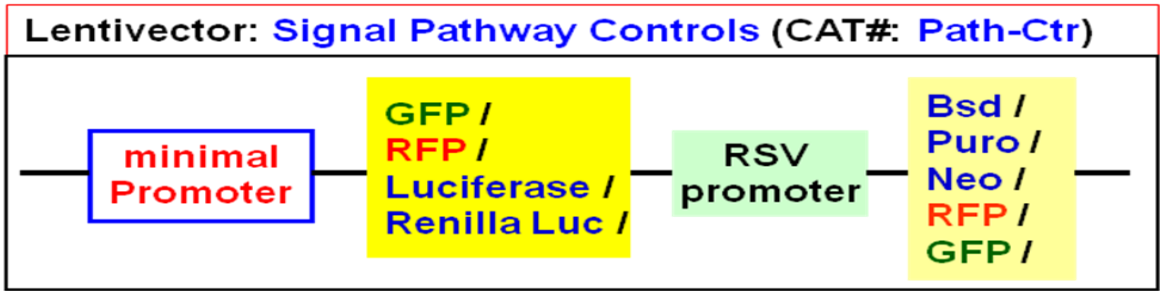
A set of **pathway Null Control lentivirus** use the minimal promoter that does not contain any signal pathway's TRE sequences and will not response to pathway's induction or treatment. The pathway control lentivirus are used to set the signal reference for the specificity of pathway treatment. See the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



The premade, ready-to-use reporter lentivirus provides an easier, sensitive and quantitative tool to monitor the activity of wnt 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.

Premade Wnt signal reporting lentivirus:

The ready-to-use lentivirus expresses a report: **firefly Luciferases (Luc), Renilla luciferase (RLuc), GFP or RFP,** under Wnt responsive promoter. The report is only expressed when the Wnt stimulating signal is present. Each lentivirus product also contains a constitutively expressed selectable marker: Blasticidin (**Bsd**), Puromycin (**Puro**), Neomycin (**Neo**), **GFP** or **RFP** fluorescent, under a separated RSV promoter.

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.

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 wnt 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.

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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.

Ready-to-use luciferase lentiviral particles are 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 is good for any cell types that requires non-serum in the medium, or good for hard-to-infect cell types.

   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% (It equivalents to a MOI=50 for most cell types at such conditions). After 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy. 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 x 10^5/ml x 0.5ml in a well of a 24-well plate.

Day 1:

- Thaw the lentivirus products at room temperature and add the appropriate amount of virus stock to obtain the desired MOI. If desired, set up the controls by using Path-control lentivirus.
- Return cells to 37°C, CO_2 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. (Note: the Pathway Control Lentivirus serves as the non-pathway specific signal background).

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:**
   - Pathway Null response Controls (CAT#: Path-Ctr1 to Path-Ctr18): Gentarget's Pathway control lentivirus contains the minimal promoter in the same lentivector backbones. 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. Be sure to select/use the pathway control virus

in the same vector backbone, i.e. having the same antibiotic marker or a fluorescent marker.

- Positive response 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 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. Ware glove all the time at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

References:

1. PLoS One, Vol. 5, No. 2, 2010;
2. The ins and outs of Wingless signaling, Trends Cell Biol. 2004 Jan;14(1):45-53.
3. "Wnt genes". Cell 1992, 69 (7): 1073–1087.

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:

BFP filter: ~Ex380 ~Em460; CFP filter: ~Ex436 ~Em480;
GFP filter: ~Ex450-490 ~Em525; YFP filter: ~Ex500 ~Em535;
RFP filter: ~Ex545 ~Em620;

Related Products: GenTarget's pre-made lentivirus product category.

Product Category	Product Description (please click category name to see product's pages)
Human, mouse or rat ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers.

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Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP**.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla** with different antibiotic selection markers.
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.
LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing with **CRISPR**
TetR inducible expression repressor	Premade lentivirus expressin **TetR** (tetracycline regulator) protein, the repressor protein for the inducible expression system.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers
T-antigen Expression	Express **SV40 large T antigen** with different selection markers
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent marker **GFP/RFP/CFP was sub-cellular localized** in different cell organelle for living cell imaging.
LacZ expression	Express different full length **β- galactosidase (lacZ)** with different selection markers
Anti-miNA lentivirus	Pre-made lentivirus expression a specific **anti-miRNA** cassette.
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
Pre-made shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more).
microRNA and anti-microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
Negative control lentiviruses	Premade **negative control lentivirus with different markers**: serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme	Ready-to-use lentivirus, expressing **specific enzymes** with different selection markers.

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expression	