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Pre-made reporting lentivirus for Wnt signaling 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 (that contains 8xTcf tandem repeats as the transcriptional response element). The Tcf repress the report expression in the absence of the Wnt signal/inducer. Once the inducer (like Wnt3A protein) is present, the luminescent or fluorescent report is expressed, which can be easily readout via luciferase assay or by fluorescent microscope. Those reporting lentivirus also contains a different fluorescent selection marker or an antibiotic selection marker under a constitutive RSV promoter, which make it easier to select the stably infected Wnt signal reporting cells (like generate WNT sensor cell lines).

Those reporting lentivirus products provide the efficient and easy tools to probe and manipulate Wnt signaling.

Premade Wnt signal reporting lentivirus:

The ready-to-use lentivirus expresses a report (**firefly Luciferases, Renilla luciferase, GFP or RFP)** under Wnt responsive promoter. The report is only expressed when the Wnt stimulating signal is present. Each lentivirus product contains a constitutively expressed selectable marker (Fluorescent or antibiotic) under a separated RSV promoter. See the lentivector core cassette in below scheme.

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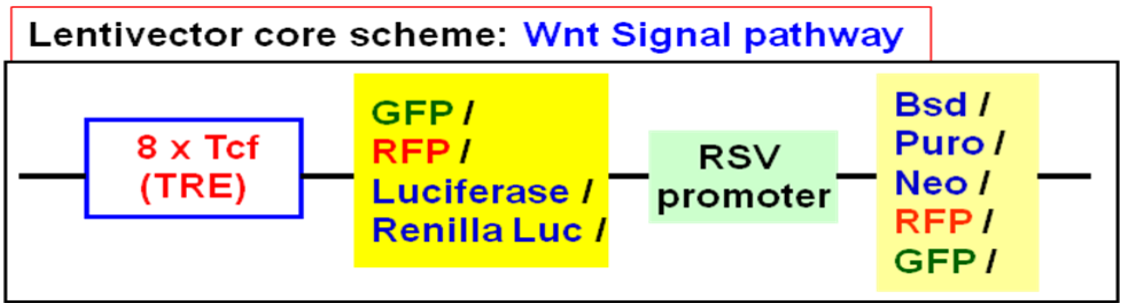

Lentivirus was pseudotyped with VSVG envelope protein, produced in 293T cells. All particles were tested to be free bacterial and mycoplasma contamination. Virus titers were tested lot by lot.

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. Or simply go for wnt signal induction without the selection. The readout can be easily monitored by luciferase assay or via the Fluorescent microscope or F-readers depending on product report type.

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:

1) Transduction Protocol for Adhesive cells :

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. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection.

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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:

- 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, CO_2 incubator.

Note: Try to avoid freezing and thawing. If you do not use all of the 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).

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).

2) Transduction Protocol for Suspension Cells:

Grow cells in complete suspension culture medium; use a shaking flask in a CO^2 incubator if necessary.

Measure cell density. When density has reached ~3 x 10^6 cells/ml, measured viability should be > 90%. Dilute cells into 1 x 10^6 cell/ml in complete medium.

Day 1:

- Thaw lentiviral particles at room temperature.
- Add premade lentiviral particles into the diluted cells at a ratio of: 50 to 100 µl virus per 0.5 ml of cells (Note: depending on cell type, you may need to use more or less virus).
- Grow cells in a shaking flask in a CO2 incubator.

Day 2:

At 24 hours after transduction, add an equal amount of fresh medium containing relevant antibiotics. **Note:** amount of antibiotic depends on cell type. Continue growing cells in CO2 incubator.

Day 3:

At 72 hours after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

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.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP**.

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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 expression	Ready-to-use lentivirus, expressing **specific enzymes** with different selection markers.