



Pre-made Reporter Lentivirus for Hedgehog Signal Pathway

Cat#	Product Name	Amounts
LVP945-P or: LVP945-P-PBS	GLI- GFP (Puro) Lentivirus	200ul, ~1 x 10 ⁷ IFU/mL in DMEM containing 10x polybrene Or 200ul, ~1 x 10 ⁸ IFU/mL in PBS solution
LVP946-P or: LVP946-P-PBS	GLI- RFP (Puro) Lentivirus	
LVP947-P or: LVP947-P-PBS	GLI- Luc (Puro) Lentivirus	
LVP948-P or: LVP948-P-PBS	GLI- Rluc (Puro) Lentivirus	
LVP1268 or: LVP1268-PBS	GLI- SEAP (Puro) Lentivirus	
LVP945-B or: LVP945-B-PBS	GLI- GFP (Bsd) Lentivirus	
LVP946-B or: LVP946-B-PBS	GLI- RFP (Bsd) Lentivirus	
LVP947-B or: LVP947-B-PBS	GLI- Luc (Bsd) Lentivirus	
LVP948-B or: LVP948-B-PBS	GLI- Rluc (Bsd) Lentivirus	
LVP1269 or: LVP1269-PBS	GLI- SEAP (Bsd) Lentivirus	
LVP945-N or: LVP945-N-PBS	GLI- GFP (Neo) Lentivirus	
LVP946-N or: LVP946-N-PBS	GLI- RFP (Neo) Lentivirus	
LVP947-N or: LVP947-N-PBS	GLI- Luc (Neo) Lentivirus	
LVP948-N or: LVP948-N-PBS	GLI- Rluc (Neo) Lentivirus	
LVP1270 or: LVP1270-PBS	GLI- SEAP (Neo) Lentivirus	
LVP945-R or: LVP945-R-PBS	GLI- GFP (RFP) Lentivirus	
LVP947-R or: LVP947-R-PBS	GLI- Luc (RFP) Lentivirus	
LVP948-R or: LVP948-R-PBS	GLI- Rluc (RFP) Lentivirus	



LVP1271 or: LVP1271-PBS	GLI- SEAP (RFP) Lentivirus	
LVP946-G or: LVP946-G-PBS	GLI- RFP (GFP) Lentivirus	
LVP947-G or: LVP947-G-PBS	GLI- Luc (GFP) Lentivirus	
LVP948-G or: LVP948-G-PBS	GLI- 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.

Hedgehog signaling pathways:

The hedgehog pathway involves stem cell growth, many type tumor growth and CNS development. The pathway is equally important during vertebrate embryonic development. The pathway takes its name from its polypeptide ligand, an intercellular signaling molecule called Hedgehog. The mammalian hedgehog has three homologues, Sonic Hedgehog (Shh), Indian Hedgehog (Ihh), and Desert Hedgehog (Dhh). The Hedgehog binds to their membrane receptor, activating the Gli proteins (transcription factors). The Gli proteins bind to Gli responsive element (Gli-RE) sequence in target's promoter region, which leads to hedgehog signal's target gene expression.

Product Principle:

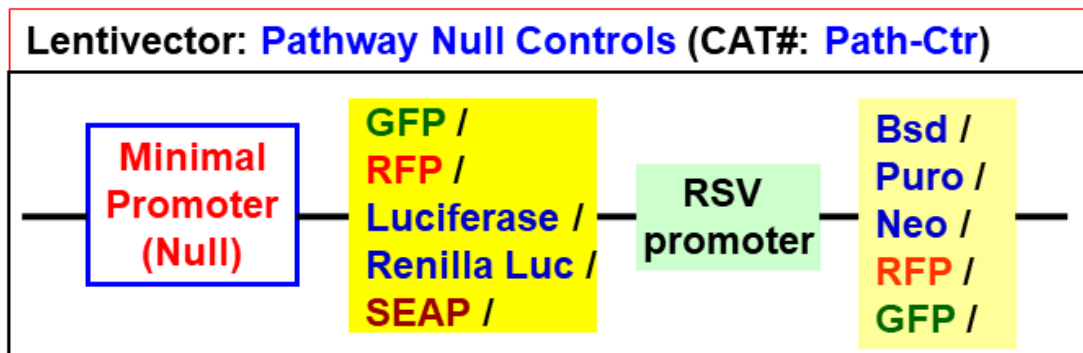
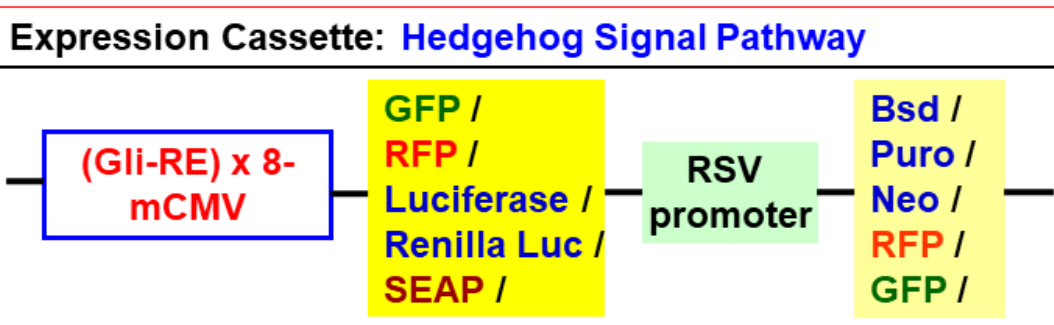
GenTarget developed a set of reporting lentivirus to measure the activation of Hedgehog signal pathway. Those reporting lentivirus has a **luminescent report (Luciferase, Renilla Luc)** or a **fluorescent report (GFP, RFP)** or a secreted **SEAP** report, under a minimal CMV promoter (mCMV) that embedded with optimized tandem repeats of Gli responsive element sequence (5'-**GACCACCCAC**). When Gli proteins bind to Gli responsive element sequence, the downstream reporter is expressed as the result of activation for the minimal



promoter. The reporter's signal can be easily and rapidly readout via plate assays (luciferase assay or by fluorescent microscope).

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 lentivector's core expression cassette scheme below.





The premade, ready-to-use reporter lentivirus provides a much easier tool to monitor the activity of Hedgehog 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 (such as Shh protein) 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 μ l 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 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:

- Nature Reviews Genetics. 2011; 12 (6): 393–406.
- Developmental Biology. 2008; 313 (2): 501–18
- Human Molecular Genetics. 2006; 15 (13): 2031–7

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;

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

Product Category	Product Description (please click category name to see product's pages)
Pathway Reporter	Lentivirus for all kinds of pathway assays
Cell Immortalization	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.
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
Human and mouse ORFs	Premade lentivirus expressing a human, mouse or rat gene with RFP-Blasticidin fusion dual markers.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression: firefly and Renilla with different antibiotic selection markers.
Fluorescent Markers	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.
CRE recombinase	Premade lentivirus for expressing nuclear permeant CRE recombinase with different fluorescent and antibiotic



	markers.
SEAP Reporter	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
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 humanized wild-type Cas9 endonuclease for genomic editing with CRISPR
TetR 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 antibiotic 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 lentiviruses 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.



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