GenTarget Inc

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Pre-made Reporter Lentivirus for Hematopoietic Cells

Cat#	Product Name	Amounts
LVP1005-P or: LVP1005-P-PBS	CD45-**GFP** (Puro) Lentivirus	200ul, 1 x 10^7 IFU/mL in DMEM medium Or 200ul, 1 x 10^8 IFU/mL in PBS solution
LVP1006-P or: LVP1006-P-PBS	CD45-**RFP** (Puro) Lentivirus	
LVP1007-P or: LVP1007-P-PBS	CD45-**Luciferase** (Puro) Lentivirus	
LVP1008-P or: LVP1008-P-PBS	CD45-**Rluc** (Puro) Lentivirus	
LVP1005-B or: LVP1005-B-PBS	CD45- **GFP** (Bsd) Lentivirus	
LVP1006-B or: LVP1006-B-PBS	CD45- **RFP** (Bsd) Lentivirus	
LVP1007-B or: LVP1007-B-PBS	CD45- **Luciferase** (Bsd) Lentivirus	
LVP1008-B or: LVP1008-B-PBS	CD45- **Rluc** (Bsd) Lentivirus	
LVP1005-N or: LVP1005-N-PBS	CD45- **GFP** (Neo) Lentivirus	
LVP1006-N or: LVP1006-N-PBS	CD45- **RFP** (Neo) Lentivirus	
LVP1007-N or: LVP1007-N-PBS	CD45- **Luciferase** (Neo) Lentivirus	
LVP1008-N or: LVP1008-N-PBS	CD45- **Rluc** (Neo) Lentivirus	
LVP1005-R or: LVP1005-R-PBS	CD45- **GFP** (RFP) Lentivirus	
LVP1007-R or: LVP1007-R-PBS	CD45- **Luciferase** (RFP) Lentivirus	
LVP1008-R or: LVP1008-R-PBS	CD45- **Rluc** (RFP) Lentivirus	
LVP1006-G or: LVP1006-G-PBS	CD45- **RFP** (GFP) Lentivirus	
LVP1007-G or: LVP1007-G-PBS	CD45- **Luciferase** (GFP) Lentivirus	
LVP1008-G or: LVP1008-G-PBS	CD45- **Rluc** (GFP) Lentivirus	
LVP1541	SFFV-**GFP** (Puro) Lentivirus in PBS	1x10^8 IFU/ml x 200ul
LVP1542	SFFV-**RFP** (Puro) Lentivirus in PBS	1x10^8 IFU/ml x 200ul
LVP1543	SFFV-**Luciferase** (Puro) Lentivirus in PBS	1x10^8 IFU/ml x 200ul
LVP1544	SFFV-**Rluc** (Puro) Lentivirus in PBS	1x10^8 IFU/ml x 200ul
LVP1545	SFFV-**SEAP** (Puro) Lentivirus in PBS	1x10^8 IFU/ml x 200ul

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

Introduction:

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.

CD45 Promoter:

PTPRC (Protein tyrosine phosphatase, receptor type, C) is also known as CD45 antigen which was originally called leukocyte common antigen (LCA). CD45 is a high molecular weight, transmembrane protein-tyrosine phosphatase expressed on all nucleated cells of hematopoietic origin. CD45's promoter is active only after ES cells differentiate into hematopoietic cell and active exclusively in all hematopoietic cells. The CD45 promoter does not contain an apparent TATA box or CCAAT box.

SFFV promoter:

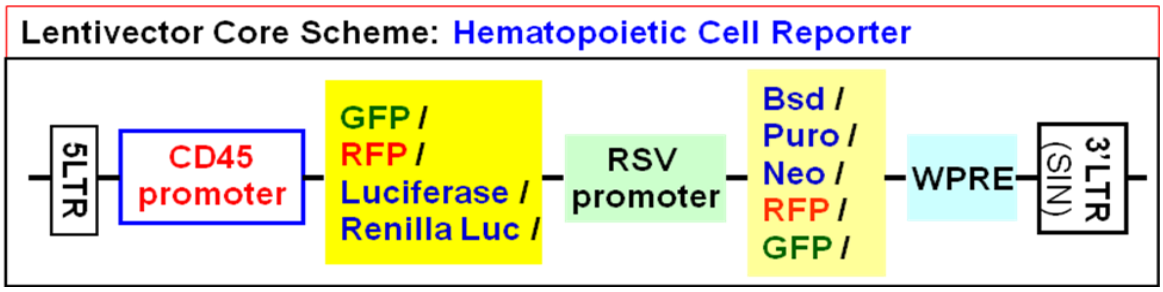
The spleen focus-forming virus (SFFV) promoter is derived from the SFFV retrovirus and is known for its strong and constitutive activity in a wide range of cell types. It is often used in gene therapy constructs to drive high levels of gene expression in hematopoietic stem cells.

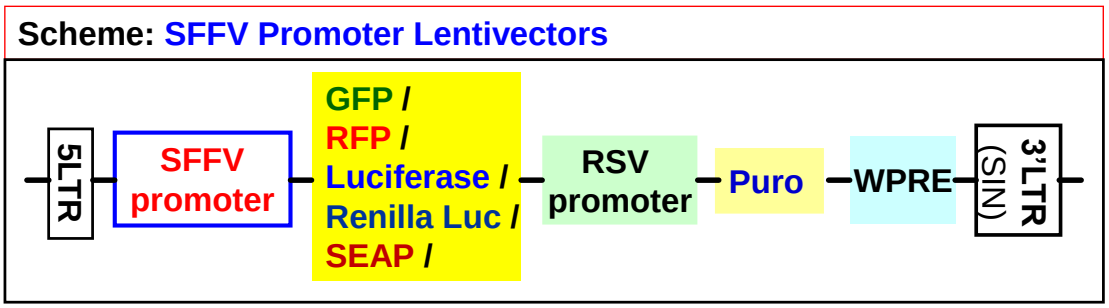
Product Principle:

GenTarget developed a set of reporting lentivirus for specifically labeling Hematopoietic cells. Those reporting lentivirus has a **luminescent report** or a **fluorescent report** under the **native promoter of human CD45 gene, or SFFV promoter** for high expression in Hematopoietic cells. Those reporter lentiviruses are best suitable for infecting the human or mouse Hematopoietic cells, as well as for the signal pathway research on CD45 promoter regulation.

Those reporting lentivirus also constitutively express a fluorescent selection marker or an antibiotic selection marker under the RSV promoter (Rous Sarcoma Virus Promoter) which is a moderate to strong promoter in most cell types. This selection marker is used to select the lentivirus infected cells (to generate the stable cell lines) via antibiotic killing or fluorescent cell

sorting. It also provides internal reference for virus transduction efficiency when a fluorescent marker is under the RSV promoter (wherever the RSV promoter is active in assay cell type). See the scheme below for lentivector's core expression cassette.





The premade, ready-to-use reporter lentivirus provides a much easier tool to specifically labeling or reporting for human or mouse Hematopoietic cells *in vitro* and *in vivo* via the luciferase signal or fluorescent signal.

Lentivirus are HIV-based, pseudotyped with VSVG envelope protein, produced in 293T cells with the 3rd generation lentivirus Bio-safety features. All particles were tested to be free bacterial and mycoplasma contamination. Virus titers were tested lot by lot.

Key Application for cell specific reporter Lentivirus:

1. Label specific cell type or create specific reporter cell line which provide a tool to monitor the specific cell type in vitro and in vivo;
2. measure the specific promoter strength in different cell types;
3. Signal pathway research on specific promoter regulation.

Product Formats:

The pre-made lentivirus provided in two formats:

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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 x 10^5/ml x 0.5ml in a well of a 24-well plate.

Day 1:

- 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 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:

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At 2 to 5 days (depends upon promoter and cell types) after transduction, check the fluorescent signal under fluorescence microscopy or by flow cytometry (FACS or Guava), or measure the luciferase activity via luciferase assay.

Day 3 +:

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 in vitro or in vivo application as pooled or single colony selcted 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:

- Experimental Hematology 42(9) · May 2014.
- Int Immunol. 1994 Aug;6(8):1279-83.
- The Journal of Biological Chemistry, June 8, 2001, 276, 19913-19920.

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.

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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.
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/ TRαV3-F2A-TRβV5-6;
CRISPR Gene Editing	Preamde lentivirus express humanzied 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	Llentivirus 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 verifeid 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.

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Anti-miNA 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.
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 fusioned 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 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 Lentiviurs 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 flurescent and antibiotic markers.
CRE, Flp ColorSwtich	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),

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TetR Repressor	Premade lentivirus expressin 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 inducible system.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic 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 lentivurs 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.