GenTarget Inc
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Pre-made Reporter Lentivirus for Megakaryocytes

Cat#	Product Name	Amounts
LVP1017-P or: LVP1017-P-PBS	ICAM2 -**GFP** (Puro) Lentivirus	200ul, ~1 x 10^7 IFU/mL in DMEM containing 10% FBS Or 200ul, ~1 x 10^8 IFU/mL in PBS solution
LVP1018-P or: LVP1018-P-PBS	ICAM2 -**RFP** (Puro) Lentivirus	
LVP1019-P or: LVP1019-P-PBS	ICAM2 -**Luciferase** (Puro) Lentivirus	
LVP1020-P or: LVP1020-P-PBS	ICAM2 -**Rluc** (Puro) Lentivirus	
LVP1017-B or: LVP1017-B-PBS	ICAM2 - **GFP** (Bsd) Lentivirus	
LVP1018-B or: LVP1018-B-PBS	ICAM2 - **RFP** (Bsd) Lentivirus	
LVP1019-B or: LVP1019-B-PBS	ICAM2 - **Luciferase** (Bsd) Lentivirus	
LVP1020-B or: LVP1020-B-PBS	ICAM2 - **Rluc** (Bsd) Lentivirus	
LVP1017-N or: LVP1017-N-PBS	ICAM2 - **GFP** (Neo) Lentivirus	
LVP1018-N or: LVP1018-N-PBS	ICAM2 - **RFP** (Neo) Lentivirus	
LVP1019-N or: LVP1019-N-PBS	ICAM2 - **Luciferase** (Neo) Lentivirus	
LVP1020-N or: LVP1020-N-PBS	ICAM2 - **Rluc** (Neo) Lentivirus	
LVP1017-R or: LVP1017-R-PBS	ICAM2 - **GFP** (RFP) Lentivirus	
LVP1019-R or: LVP1019-R-PBS	ICAM2 - **Luciferase** (RFP) Lentivirus	
LVP1020-R or: LVP1020-R-PBS	ICAM2 - **Rluc** (RFP) Lentivirus	
LVP1018-G or: LVP1018-G-PBS	ICAM2 - **RFP** (GFP) Lentivirus	
LVP1019-G or: LVP1019-G-PBS	ICAM2 - **Luciferase** (GFP) Lentivirus	
LVP1020-G or: LVP1020-G-PBS	ICAM2 - **Rluc** (GFP) Lentivirus	

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

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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.

ICAM2 Promoter:

Intercellular adhesion molecule 2 (ICAM2), also known as CD102, is a cell surface adhesion molecule constitutively expressed in vasculature endothelial cells and megakaryocytes, involved in leukocyte recruitment into tissues. Its promoter contains four transcription factor binding sites such as Sp1 and GATA motifs but no TATA box. Human ICAM-2 promoter contains all of the signals necessary for endothelium-specific transgene expression. The promoter activity is down-regulated by TNF-α. Reporter signal driven by this promoter was 125-fold greater in bovine aortic endothelial cells but less than 2-fold in non-endothelial (COS) cells, contrast to background.

Product Principle:

GenTarget developed a set of reporting lentivirus for specifically labeling vasculature endothelial cells and megakaryocytes. Those reporting lentivirus has a **luminescent report** or a **fluorescent report** under the **native promoter of human ICAM2 gene** that constitutively expressed in vasculature endothelial cells and megakaryocytes. Those reporter lentivirus are best suitable for infecting the human or mouse vasculature endothelial cells and megakaryocytes, as well as for the signal pathway research on ICAM2 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.

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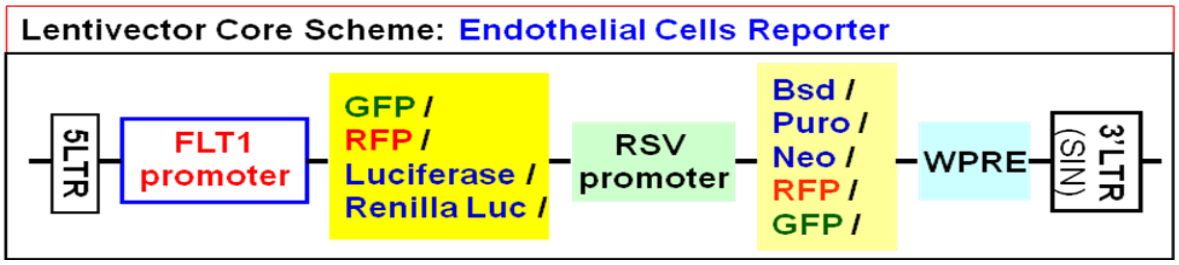

The premade, ready-to-use reporter lentivirus provides a much easier tool to specifically labeling or reporting for human or mouse vasculature endothelial cells and megakaryocytes *in vitro* and *in vivo* via the luciferase signal or fluorescent signal.

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:

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:

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:

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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:

- Journal of Cell Science 112, 4695-4703 (1999).
- J Biol Chem. 1998, 273(19):11737-44.

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 categories:

Lentivirus Category (click to see)	**Product Description**
Target Expression	Premade lentivirus express a **human, mouse or rat** gene with Fluorescent-Antibiotic fusion dual selection.
Luciferase expression	Premade lentivirus express all kinds of luciferase**: firefly; Renilla; Cypridina; Red-Luc; Nano-Luc,** with different fluorescent and antibiotic selection.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP/niRFP /unstable GFP, etc.**
Cytoskeleton Imaging	Fluorescent (GFP / RFP/ CFP) labelled cell skeleton protein (Actin; Tubulin; Paxillin; Vimentin)
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent labelled cell organelle lentivirus for living cell imaging.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing by **CRISPR**
Fluorescent Fusion target	Lentivirus express the "**Fluorescent-Target**" fusion proteins. A desired target is fused to Green, Blue, Red, or Cyan

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	Fluorescent Protein, demonstrating the target's functionality and localization
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.
SEAP Reporter	**SEAP** (Secreted Embryonic Alkaline Phosphatase) secreted expression lentivirus under different promoter.
TetR repressor expression	Premade lentivirus expressin **TetR** (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	Lentivirus express the reverse tetraccycline transcription activator gene, rtTA-M2 with different selection.
Pathway Reporter	Different Report lentivirus (**Luc, RFP, GFP, SEAP**) under a pathway specific response promoter.
Cell Immortalization	Comprehesive lentivirus for cell immortalization, for different cell types.
Cell Specific reporter	Different Report lentivirus driven by cell specific promoter.
Infectious Antigens	Lentivirus express all kinds of infectious antigens.
Viral Like Particle (VLP)	Lentiviral particles pseudo-typed with high density of surface envelope protein.
Immuno Therapy	Lentivirus products for Immuno Therapy application.
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
Anti-miNA lentivirus	Pre-made lentivirus expression a specific **anti-miRNA** cassette.
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

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