

7930 Arjons Drive, Suite B San Diego, CA 92126, USA Phone: 1 (858) 265-6446

Fax: 1 (800) 380-4198 Email: orders@gentarget.com

Pre-made Reporter Lentivirus for Prostate Cells

Cat#	Product Name	Amounts
<u>LVP1045-P</u> <u>or: LVP1045-P-PBS</u>	PSA - GFP (<mark>Puro</mark>) Lentivirus	
<u>LVP1046-P</u> <u>or:</u> LVP1046-P-PBS	PSA - RFP (<mark>Puro</mark>) Lentivirus	
LVP1047-P or: LVP1047-P-PBS	PSA - Luciferase (Puro) Lentivirus	
LVP1048-P or: LVP1048-P-PBS	PSA - Rluc (Puro) Lentivirus	
<u>LVP1045-B</u> or: LVP1045-B-PBS	PSA - GFP (Bsd) Lentivirus	
<u>LVP1046-B</u> or: LVP1046-B-PBS	PSA - RFP (Bsd) Lentivirus	
LVP1047-B or: LVP1047-B-PBS	PSA - Luciferase (Bsd) Lentivirus	200ul, ~1 x 10 ⁷ IFU/mL
LVP1048-B or: LVP1048-B-PBS	PSA - Rluc (Bsd) Lentivirus	in DMEM containing 10% FBS
LVP1045-N or: LVP1045-N-PBS	PSA - GFP (Neo) Lentivirus	Or
LVP1046-N or: LVP1046-N-PBS	PSA - RFP (Neo) Lentivirus	200ul, ~1 x 10 ⁸ IFU/mL
LVP1047-N or: LVP1047-N-PBS	PSA - Luciferase (Neo) Lentivirus	in PBS solution
LVP1048-N or: LVP1048-N-PBS	PSA - Rluc (Neo) Lentivirus	
LVP1045-R or: LVP1045-R-PBS	PSA - GFP (RFP) Lentivirus	
LVP1047-R or: LVP1047-R-PBS	PSA - Luciferase (RFP) Lentivirus	
<u>LVP1048-R</u> or: LVP1048-R-PBS	PSA - Rluc (RFP) Lentivirus	
<u>LVP1046-G</u> or: LVP1046-G-PBS	PSA - RFP (GFP) Lentivirus	
LVP1047-G or: LVP1047-G-PBS	PSA - Luciferase (GFP) Lentivirus	
LVP1048-G or: LVP1048-G-PBS	PSA - 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 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.

PSA Promoter:

Prostate-specific antigen (PSA) is secreted by the epithelial cells of the prostate gland. PSA is expressed at very low level in healthy prostate, but is often elevated in the presence of prostate cancer or other prostate disorders. PSA is a serum marker for prostate cancer.

PSA promoter contains the androgen response elements (AREs). PSA expression is tightly regulated by androgen. Upon binding to androgen, androgen receptor (AR) translocates into the nucleus and binds to the PSA promoter's AREs, where it interacts with other transcription factors and activates PSA gene transcription. PSA promoter activity is known to be highly prostate tissue or cell-specific.

Product Principle:

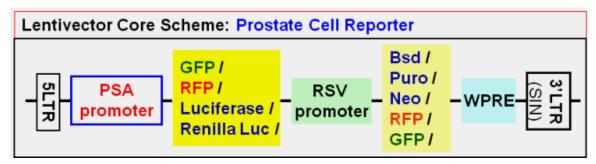
GenTarget developed a set of reporting lentivirus for selectively labeling prostate cancer cells. Those reporting lentivirus has a **luminescent report** or a **fluorescent report** under the **native promoter of human PSA gene** that specifically expressed in prostate tissue or cells. Those reporter lentivirus are best suitable for infecting the human or mouse prostate cells or prostate cancer, as well as for the signal pathway research on PSA 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 services as 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 prostate 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:

- 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.



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For more details about premade particles, please see <u>FAQ for pre-made</u> <u>lentiviral particles</u> (.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 $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$ 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, 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 $^{\circ}$ 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 selected 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 Biosafety 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:

- The Journal of Urology. 1994, 151 (5): 1283–90.
- The Journal of Biological Chemistry. 2000,275, 40846-40855.
- Cancer Res. 2000 Jan 15;60(2):334-41.
- Cancer Gene Therapy. 2001, Volume 8, Number 9, Pages 628-635

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.

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;



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Product	Product Description	
Category	(please click into each category's page)	
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.	
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.	
<u>Luciferase</u> <u>expression</u>	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.	



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Product	Product Description
Category	(please click into each category's page)
Luminescent	Lentivirus express Nano-Latern as Bio-probes for in vivo
<u>Imaging</u>	imaging of sub-cellular structural organization and
	dynamic processes in living cells and organisms
<u>Sub-cellular</u>	Lentivirus contain a well-defined organelle targeting
<u>Imaging</u>	signal fusioned to a fluorescent protein, great tools for
	live-cell imaging and for dynamic investigation of sub-
	cellular signal pathways.
<u>Cytoskeleton</u>	A fluorescent marker (GFP, RFP or CFP) fusion with a
<u>Imaging</u>	cellular structure protein, provides a convenient tool for
	visualization of cytoskeletal structure
<u>Unstable GFP</u>	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	Pre-made lentivirus expression a "GFP/RFP/CFP-ORF"
fusion	fusion target.
	Premade lentivirus for expressing nuclear permeant
CRE recombinase	CRE recombinase with different flurescent and antibiotic
	markers.
CRE, Flp	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or
<u>ColorSwtich</u>	"FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the
	CRE or Flp recombination event in vivo.
	lentivirus expressing SEAP under different promoters
SEAP Reporter	(TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal
	pathway responsive promoter),
	Premade lentivirus expressin TetR (tetracycline
<u>TetR Repressor</u>	regulator) protein, the repressor protein for the
	inducible expression system.
	rtTA binds to the tetracycline operator element (TetO) in
rtTA Expression	the presence of doxycycline (Dox). Used for Tet-On /OFF
	inducible system.
:DC 6 .	Premde lentivirus for human and mouse iPS (Myc,
<u>iPS factors</u>	NANOG, OCT4, SOX2, FLF4) factors with different
	fluorescent and antibitoic markers
<u>LacZ expression</u>	Express different full length β- galactosidase
	(lacZ) with different selection markers



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Product Category	Product Description (please click into each category's page)
	Premade negative control lentivirus with different markers : serves as the negative control of lentivurs
Negative control lentiviruses	treatment, for validation of the specificity of any
	lentivirus target expression effects.
Other Enzyme	Ready-to-use lentivirus, expressing a specific enzymes
<u>expression</u>	with different selection markers.
<u>Ultra titer</u>	Ultra-titer lentivirus used for the hard-to-transduced
<u>lentivirus</u>	cells and for in vivo manipulation of sperm cells, or stem
	cells.