



## Reporting lentivirus driven by heat inducible promoter

Cat#	Product Name	Amounts
<a href="#">LVP863-P-PBS</a>	Hsp70- <b>GFP</b> ( <b>Puro</b> ) Lentivirus	200ul/each (1 x 10 <sup>8</sup> IFU/mL), containing 10x of Polybrene
<a href="#">LVP864-P-PBS</a>	Hsp70- <b>RFP</b> ( <b>Puro</b> ) Lentivirus	
<a href="#">LVP865-P-PBS</a>	Hsp70- <b>Firefly Luc</b> ( <b>Puro</b> ) Lentivirus	
<a href="#">LVP866-P-PBS</a>	Hsp70- <b>Renilla Luc</b> ( <b>Puro</b> ) Lentivirus	
<a href="#">LVP863-B-PBS</a>	Hsp70 <b>GFP</b> ( <b>Bsd</b> ) Lentivirus	
<a href="#">LVP864-B-PBS</a>	Hsp70- <b>RFP</b> ( <b>Bsd</b> ) Lentivirus	
<a href="#">LVP865-B-PBS</a>	Hsp70- <b>Firefly Luc</b> ( <b>Bsd</b> ) Lentivirus	
<a href="#">LVP866-B-PBS</a>	Hsp70- <b>Renilla Luc</b> ( <b>Bsd</b> ) Lentivirus	
<a href="#">LVP863-N-PBS</a>	Hsp70- <b>GFP</b> (Neo) Lentivirus	
<a href="#">LVP864-N-PBS</a>	Hsp70- <b>RFP</b> (Neo) Lentivirus	
<a href="#">LVP865-N-PBS</a>	Hsp70- <b>Firefly Luc</b> (Neo) Lentivirus	
<a href="#">LVP866-N-PBS</a>	Hsp70- <b>Renilla Luc</b> (Neo) Lentivirus	
<a href="#">LVP863-R-PBS</a>	Hsp70- <b>GFP</b> ( <b>RFP</b> ) Lentivirus	
<a href="#">LVP865-R-PBS</a>	Hsp70- <b>Firefly Luc</b> ( <b>RFP</b> ) Lentivirus	
<a href="#">LVP866-R-PBS</a>	Hsp70- <b>Renilla Luc</b> ( <b>RFP</b> ) Lentivirus	
<a href="#">LVP864-G-PBS</a>	Hsp70- <b>RFP</b> ( <b>GFP</b> ) Lentivirus	
<a href="#">LVP865-G-PBS</a>	Hsp70- <b>Firefly Luc</b> ( <b>GFP</b> ) Lentivirus	
<a href="#">LVP866-G-PBS</a>	Hsp70- <b>Renilla Luc</b> ( <b>GFP</b> ) Lentivirus	
<a href="#">LVP816-PBS</a>	Hsp70- <b>Luc</b> / <b>GFP</b> ( <b>RFP</b> - <b>Bsd</b> ) lentivirus	

**Storage:** -80 °C, avoid repeat freeze/thaw cycles. Stable for 12 months.

### 1. 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.

Hsp70 promoter is a heat inducible promoter that drives the Heat Shock Protein-70 expression. Heat Shock Proteins are a family of conserved ubiquitously expressed proteins that are an important part of the cell's machinery for protein folding, and help to protect cells from stress. The Heat shock proteins are strongly up-regulated by heat stress and toxic chemicals, particularly heavy metals such as arsenic, cadmium, copper, mercury, and near infrared light, etc.

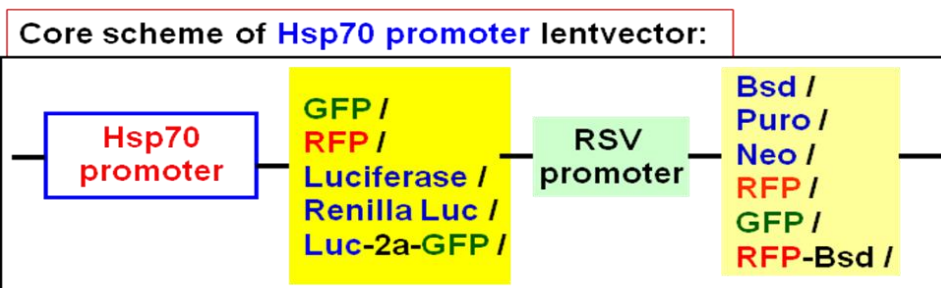


The **HSP70 promoter** contains at least two regulatory domains, a distal domain responsive to heat shock or heavy metals and a proximal domain responsive to stimulation by serum. The activity of the HSP70 promoter can be induced by moderate hyperthermia (39°C to 43°C), reaching expression levels similar to those of the CMV promoter.

## 2. Premade Heat inducible reporting lentivirus:

Gentarget created a set of reporting lentivirus in which a report (**firefly Luciferases, Renilla luciferase, GFP or RFP**) is driven by Hsp70 promoter. The report's expression is moderate at normal culture condition and up-regulated upon the stimulation, such as hyperthermia (42 °C) for 1 hour. The promoter activation reaches to its peak at about 24 h after heat shock and could be maintained for 3 days with a gradual decline.

Those products also contain a constitutively expressed antibiotic selection marker for positive cell selection, or a 2nd fluorescent marker which serves as internal normalization control. See core lentivector scheme below.



Those reporting lentivirus products provide the efficient and easy tools for researching or monitoring Hsp promoter strength under all kinds of stimulation, such a metal ion, near-infrared light illumination and heating treatment, and provide the temporal and spatial control of gene expression.

For more details about premade particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf).

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

## 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, CO<sub>2</sub> 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).

**For report inducible expression, *In vitro*:** put the cell culture plates or the collected cells under 42°C incubator or water bath, accordingly, for 1 hour, and put cell plate (or seed cell into culture plate) back to 37°C, CO<sub>2</sub> continues incubation. The heat induced expression will peak at 24 hour post heat treatment. *In Vivo*: use near-infrared light or other desired methods to achieve the heat treatment.

## 4. 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. Wear glove all the time at handling Lentivirus! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

## 5. References:

Vaccines 2014, 2, 216-227; doi:10.3390;

## 6. 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;

## 7. Attachment: GenTarget's pre-made lentivirus product categories.

<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">Pathway Reporter</a>	Reporter Lentivirus for all kinds of pathway screening assays
<a href="#">Cell Immortalization</a>	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HPV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.
<a href="#">ImmunoOncology Research</a>	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;
<a href="#">CAR-T, TCR Lentivirus</a>	<b>CARs</b> Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; <b>TCRs</b> : MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRaV3-F2A-TRβV5-6;
<a href="#">CRISPR Gene Editing</a>	Preamde lentivirus express humanized wild-type <b>Cas9</b> endonuclease, the <b>dCas9</b> , gRNAs, <b>CRISPR</b> gene editing research



<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">Epigenomic: CRISPRi and CRISPRa</a>	" <b>dCas9-Protein</b> " fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
<a href="#">Cell-Specific Reporter</a>	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
<a href="#">Infectious Antigens</a>	Lentivirus that express all kinds of infectious antigens with C-term 6His-tag.
<a href="#">Virus Like Particles (VLP)</a>	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
<a href="#">Non-integrating LV</a>	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
<a href="#">shRNA Knockdown</a>	Knockdown verified and customized shRNA lentivirus for target knockdown,
<a href="#">microRNA lentivirus</a>	Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human and mouse miRNA.
<a href="#">Anti-miRNA lentivirus</a>	Pre-made lentivirus expression a specific anti-miRNA cassette.
<a href="#">Human and mouse ORFs</a>	Premade lentivirus expressing a <b>human, mouse or rat</b> gene with RFP-Blasticidin fusion dual markers.
<a href="#">Luciferase expression</a>	Premade lentivirus for all kinds of luciferase protein expression: <b>firefly and Renilla, Red-Luc and more</b> , with different antibiotic selection markers.
<a href="#">Fluorescent Markers</a>	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, mRFP, unstable GFP and others.
<a href="#">Luminescent Imaging</a>	Lentivirus express Nano-Lantern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
<a href="#">Sub-cellular Imaging</a>	Lentivirus contain a well-defined organelle targeting signal fused to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways.



<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">Cytoskeleton Imaging</a>	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
<a href="#">Unstable GFP</a>	Lentivirus express the the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection
<a href="#">near-infrared RFP</a>	The near-infrared Red fluorescent (niRFP) expression Lentiviurs provides the whole-body images with better contrast and brighter images
<a href="#">Fluorescent-ORF fusion</a>	Pre-made lentivirus expression a " <b>GFP/RFP/CFP-ORF</b> " fusion target.
<a href="#">CRE recombinase</a>	Premade lentivirus for expressing <b>nuclear permeant CRE</b> recombinase with different flurescent and antibiotic markers.
<a href="#">CRE, Flp ColorSwich</a>	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.
<a href="#">SEAP Reporter</a>	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
<a href="#">TetR Repressor</a>	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
<a href="#">rtTA Expression</a>	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF inducible system.
<a href="#">iPS factors</a>	Premde lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FLF4</b> ) factors with different fluorescent and antibitoic markers
<a href="#">LacZ expression</a>	Express different full length <b>β- galactosidase (lacZ)</b> with different selection markers
<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme expression</a>	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.
<a href="#">Ultra titer</a>	Ultra-titer lentivirus used for the hard-to-transduced



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<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">lentivirus</a>	cells and for in vivo manipulation of sperm cells, or stem cells.