



## Pre-made reporting lentivirus driven by heat inducible promoter

Cat#	Product Name	Amounts
<a href="#">LVP863-P</a> or <a href="#">LVP863-P-PBS</a>	Hsp70- <b>GFP</b> (Puro) Lentiviral particles	200ul, ~1 x 10 <sup>7</sup> IFU/mL in DMEM containing 10% FBS  Or  200ul, ~1 x 10 <sup>8</sup> IFU/mL in PBS solution
<a href="#">LVP864-P</a> or <a href="#">LVP864-P-PBS</a>	Hsp70- <b>RFP</b> (Puro) Lentiviral particles	
<a href="#">LVP865-P</a> or <a href="#">LVP865-P-PBS</a>	Hsp70- <b>Firefly Luc</b> (Puro) Lentiviral particles	
<a href="#">LVP866-P</a> or <a href="#">LVP866-P-PBS</a>	Hsp70- <b>Renilla Luc</b> (Puro) Lentiviral particles	
<a href="#">LVP863-B</a> or <a href="#">LVP863-B-PBS</a>	Hsp70 <b>GFP</b> (Bsd) Lentiviral particles	
<a href="#">LVP864-B</a> or <a href="#">LVP864-B-PBS</a>	Hsp70- <b>RFP</b> (Bsd) Lentiviral particles	
<a href="#">LVP865-B</a> or <a href="#">LVP865-B-PBS</a>	Hsp70- <b>Firefly Luc</b> (Bsd) Lentiviral particles	
<a href="#">LVP866-B</a> or <a href="#">LVP866-B-PBS</a>	Hsp70- <b>Renilla Luc</b> (Bsd) Lentiviral particles	
<a href="#">LVP863-N</a> or <a href="#">LVP863-N-PBS</a>	Hsp70- <b>GFP</b> (Neo) Lentiviral particles	
<a href="#">LVP864-N</a> or <a href="#">LVP864-N-PBS</a>	Hsp70- <b>RFP</b> (Neo) Lentiviral particles	
<a href="#">LVP865-N</a> or <a href="#">LVP865-N-PBS</a>	Hsp70- <b>Firefly Luc</b> (Neo) Lentiviral particles	
<a href="#">LVP866-N</a> or <a href="#">LVP866-N-PBS</a>	Hsp70- <b>Renilla Luc</b> (Neo) Lentiviral particles	
<a href="#">LVP863-R</a> or <a href="#">LVP863-R-PBS</a>	Hsp70- <b>GFP</b> (RFP) Lentiviral particles	
<a href="#">LVP865-R</a> or <a href="#">LVP865-R-PBS</a>	Hsp70- <b>Firefly Luc</b> (RFP) Lentiviral particles	
<a href="#">LVP866-R</a> or <a href="#">LVP866-R-PBS</a>	Hsp70- <b>Renilla Luc</b> (RFP) Lentiviral particles	
<a href="#">LVP864-G</a> or <a href="#">LVP864-G-PBS</a>	Hsp70- <b>RFP</b> (GFP) Lentiviral particles	
<a href="#">LVP865-G</a> or <a href="#">LVP865-G-PBS</a>	Hsp70- <b>Firefly Luc</b> (GFP) Lentiviral particles	
<a href="#">LVP866-G</a> or <a href="#">LVP866-G-PBS</a>	Hsp70- <b>Renilla Luc</b> (GFP) Lentiviral particles	
<a href="#">LVP816</a> or <a href="#">LVP816-PBS</a>	Hsp70- <b>Luc / GFP</b> ( <b>RFP</b> - <b>Bsd</b> ) 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



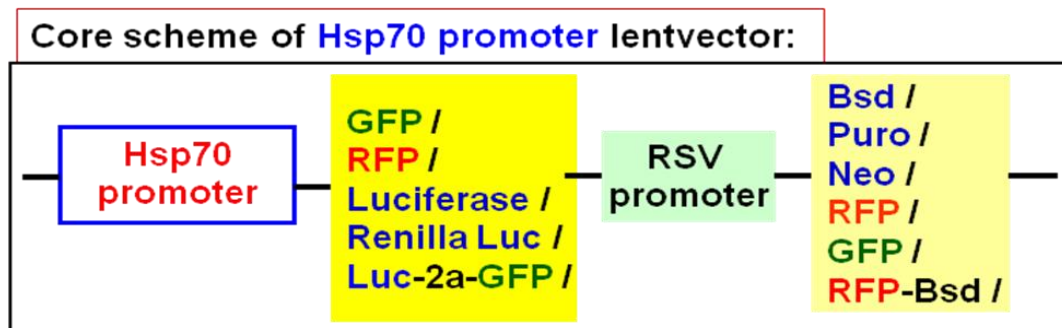
knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into almost all kinds of mammalian cells, including stem cells, primary cells, and non-dividing cells both *in vivo* and *in vitro*. Lentiviral Particles stably integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.

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.

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 oC) 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.

## **Premade Heat inducible reporting lentivirus:**

Lentivirus was 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.

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, or directly go for the heat induced expression at 2 to 3 days post virus transduction without selection of the positive transduced cells. The readout can be easily monitored by luciferase assay or via the Fluorescent microscope or Fluorescent-readers depending on product report type.

## **Ready-to-use luciferase lentiviral particles are 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 is good for any cell types that require non-serum in the medium, or good for hard-to-infect cell types.

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  $\mu$ 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. 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.

## 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 Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

## References:

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



## 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;

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

<b>Product Category</b>	<b>Product Description (please click category name to see product's pages)</b>
<a href="#">Human, mouse or rat ORFs</a>	Premade lentivirus expressing a <b>human, mouse or rat</b> gene with RFP-Blastididin fusion dual markers.
<a href="#">Fluorescent markers</a>	Preamde lentivirus express human codon optimized fluorescent protein, <b>GFP / RFP / CFP / BFP / YFP</b> .
<a href="#">Luciferase expression</a>	Premade lentivirus for all kinds of luciferase protein expression: <b>firefly and Renilla</b> with different antibiotic selection markers.
<a href="#">CRE recombinase</a>	Premade lentivirus for expressing <b>nuclear permeant CRE</b> recombinase with different fluorescent and antibiotic markers.
<a href="#">LoxP ColorSwitch</a>	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
<a href="#">CRISPR /hu CAS9</a>	Preamde lentivirus express humanized wild-type <b>Cas9</b> endonuclease for genomic editing with <b>CRISPR</b>
<a href="#">TetR inducible expression repressor</a>	Premade lentivirus expressing <b>TetR</b> (tetracycline regulator) protein, the repressor protein for the inducible expression system.
<a href="#">Pathway reporting</a>	Premade lentivirus for monitoring specific signal pathway activities and for cell based assays.
<a href="#">iPS factors</a>	Premde lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FGF4</b> ) factors with different fluorescent and antibiotic markers
<a href="#">T-antigen Expression</a>	Express <b>SV40 large T antigen</b> with different selection markers
<a href="#">Cell</a>	Premade lentivirus for cell organelle imaging. The fluorescent



<a href="#">Organelle imaging</a>	marker <b>GFP/RFP/CFP</b> was <b>sub-cellular localized</b> in different cell organelle for living cell imaging.
<a href="#">LacZ expression</a>	Express different full length <b>β-galactosidase (lacZ)</b> with different selection markers
<a href="#">Anti-miRNA lentivirus</a>	Pre-made lentivirus expression a specific <b>anti-miRNA</b> cassette.
<a href="#">Fluorescent-ORF fusion</a>	Pre-made lentivirus expression a " <b>GFP/RFP/CFP-ORF</b> " fusion target.
<a href="#">Pre-made shRNA lentivirus</a>	Premade shRNA lentivirus for knockdown a specific genes ( <b>P53, LacZ, Luciferase</b> and more).
<a href="#">microRNA and anti-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="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentiviruses treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme expression</a>	Ready-to-use lentivirus, expressing <b>specific enzymes</b> with different selection markers.