



## Mito-SypHer (pH sensitive probe) lentiviruses

Cat#	Product Name	Amounts
<a href="#">LVP1114-P</a>	Mito-SypHer (CMV-Puro) Lentivirus	<b>200ul,</b> (1 x 10 <sup>7</sup> IFU/mL, containing 10x polybrene)
<a href="#">LVP1114-B</a>	Mito-SypHer (CMV-Bsd) Lentivirus	
<a href="#">LVP1114-N</a>	Mito-SypHer (CMV-Neo) Lentivirus	
<a href="#">LVP1115-P</a>	Mito-SypHer (EF1a-Puro) Lentivirus	
<a href="#">LVP1115-B</a>	Mito-SypHer (EF1a-Bsd) Lentivirus	
<a href="#">LVP1115-N</a>	Mito-SypHer (EF1a-Neo) Lentivirus	
<a href="#">LVP1114-P-PBS</a>	Mito-SypHer (CMV-Puro) Lentivirus	<b>200ul,</b> (1 x 10 <sup>8</sup> IFU/mL, in PBS solution)
<a href="#">LVP1114-B-PBS</a>	Mito-SypHer (CMV-Bsd) Lentivirus	
<a href="#">LVP1114-N-PBS</a>	Mito-SypHer (CMV-Neo) Lentivirus	
<a href="#">LVP1115-P-PBS</a>	Mito-SypHer (EF1a-Puro) Lentivirus	
<a href="#">LVP1115-B-PBS</a>	Mito-SypHer (EF1a-Bsd) Lentivirus	
<a href="#">LVP1115-N-PBS</a>	Mito-SypHer (EF1a-Neo) Lentivirus	

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

### Product Description:

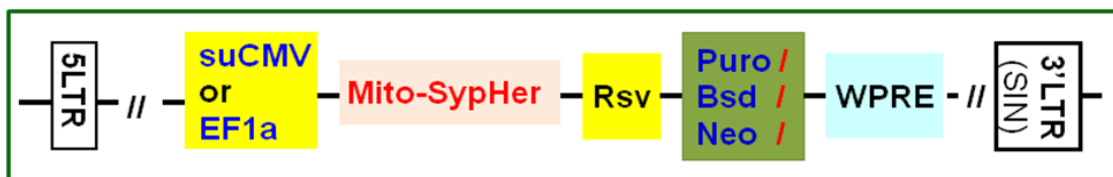
Lentiviral system is a gene delivery tool using lentivectors for gene expression or knockdown. GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced 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.

Mitochondria are double membrane organelles with an outer membrane permeable to solutes and an inner membrane harboring the respiratory chain



complexes. Mitochondria extrude protons across their inner membrane to generate pH gradient which powers ATP synthesis. SypHer is a mutated form of Hyper, a genetically encoded sensor for hydrogen peroxide. The mutations make SypHer insensitivity to H<sub>2</sub>O<sub>2</sub>. The Mito-SypHer is a mitochondrial targeted pH-sensitive probe. The Mito-SypHer provides a tool for dynamic measurement of pH changes at mitochondria in living cells. Mito-SypHer is a ratiometric probe, excited at 420/480nm with 505nm Dichroic and 535nm (+/- 25nm) Emission filter.

Gentarget created a set of expression lentivirus for Mito-SypHer probe. Those products also contain a constitutively expressed Mito-SypHer under the enhanced CMV or EF1a promoter. The CMV demonstrated the highest expression levels in most cell types. The EF1a demonstrated high expression with much less tissue specificity and without promoter-silencing effects during long-term culture. An antibiotic selection marker is also included under the RAV promoter. See core lentivector scheme below.



Those probe lentivirus products provide the efficient and easy tools for dynamical research or measurement of pH changes at mitochondria in living cells.

### **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  $0.5 \times 10^5$ /ml x 0.5ml 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, CO2 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. JBC, April 1, 2011, vol. 286 no. 13 11672-11684 ;
2. The Journal of General Physiology, 2012, Nov 1, 140(5):567
3. EMBO Reports (2017) Feb. 7, 18: 451-463

### 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.

**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>	Lentivirus for all kinds of pathway 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-T, TCR-T, Assay cell lines, and Cell Antigens & Receptors.
<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
<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="#">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="#">LoxP ColorSwitch</a>	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE 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 antibiotic markers
<a href="#">LacZ expression</a>	Express different full length <b><math>\beta</math>- 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 lentivirus</a>	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.



# GenTarget Inc

7930 Arjons Drive, Suite B  
San Diego, CA 92126, USA  
Phone: (858) 2656446  
Fax: (800) 3804198  
Email: [orders@gentarget.com](mailto:orders@gentarget.com)

---

--	--