



Mito-SypHer (pH sensitive probe) lentiviruses

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

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

Product Description:

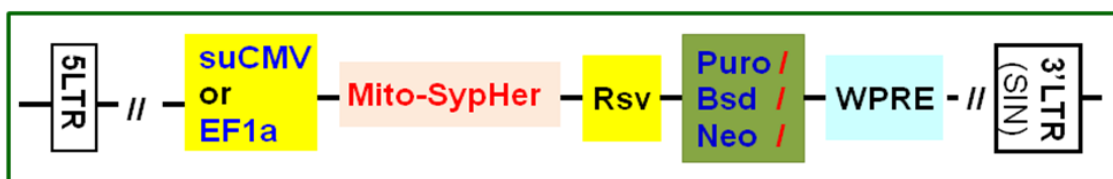
Lentiviral system is a gene delivery tool using lentivectors for gene expression or knockdown. GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced 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_2O_2 . 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 μ 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, CO₂ 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 adapt 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.

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Reporter 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,



Product Category	Product Description (please click into each category's page)
	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;
CAR-T, TCR Lentivirus	CARs Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; TCRs : MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRaV3-F2A-TRβV5-6;
CRISPR Gene Editing	Preamde lentivirus express humanized 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	Lentivirus 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 verified 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-miRNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.



Product Category	Product Description (please click into each category's page)
Human and mouse ORFs	Premade lentivirus expressin a human, mouse or rat gene with RFP-Blastididin fusion dual markers.
Luciferase expression	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.
Luminescent Imaging	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
Sub-cellular Imaging	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.
Cytoskeleton Imaging	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
Unstable GFP	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 fusion	Pre-made lentivirus expression a " GFP/RFP/CFP-ORF " fusion target.
CRE recombinase	Premade lentivirus for expressing nuclear permeant CRE recombinase with different flurescent and antibiotic markers.
CRE, Flp ColorSwich	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.
SEAP Reporter	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
TetR Repressor	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.



Product Category	Product Description (please click into each category's page)
rtTA Expression	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF inducible system.
iPS factors	Premade lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FLK4) factors with different fluorescent and antibiotic markers
LacZ expression	Express different full length β-galactosidase (lacZ) with different selection markers
Negative control lentiviruses	Premade negative control lentivirus with different markers : serves as the negative control of lentivirus treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.
Ultra titer lentivirus	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.