GenTarget Inc
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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.

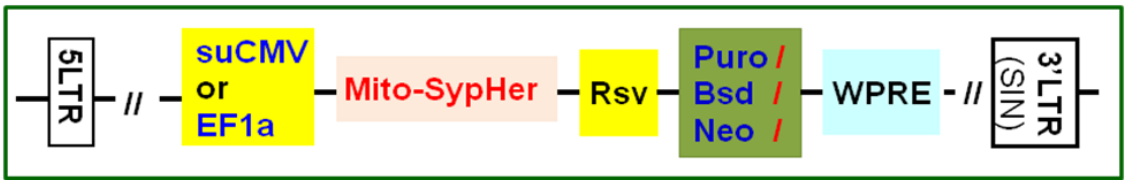
Product Description:

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:

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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 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 x 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_2 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).

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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. Ware 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.

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

Lentivirus Category (click to see)	**Product Description**
Target Expression	Premade lentivirus express a **human, mouse or rat** gene with Fluorescent-Antibiotic fusion dual selection.

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Luciferase expression	Premade lentivirus express all kinds of luciferase**: firefly; Renilla; Cypridina; Red-Luc; Nano-Luc,** with different fluorescent and antibiotic selection.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP/niRFP /unstable GFP, etc.**
Cytoskeleton Imaging	Fluorescent (GFP / RFP/ CFP) labelled cell skeleton protein (Actin; Tubulin; Paxillin; Vimentin)
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent labelled cell organelle lentivirus for living cell imaging.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing by **CRISPR**
Fluorescent Fusion target	Lentivirus express the "**Fluorescent-Target**" fusion proteins. A desired target is fused to Green, Blue, Red, or Cyan Fluorescent Protein, demonstrating the target's functionality and localization
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.
LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
SEAP Reporter	**SEAP** (Secreted Embryonic Alkaline Phosphatase) secreted expression lentivirus under different promoter.
TetR repressor expression	Premade lentivirus expressin **TetR** (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	Lentivirus express the reverse tetraccycline transcription activator gene, rtTA-M2 with different selection.
Pathway Reporter	Different Report lentivirus (**Luc, RFP, GFP, SEAP**) under a pathway specific response promoter.
Cell Immortalization	Comprehesive lentivirus for cell immortalization, for different cell types.
Cell Specific reporter	Different Report lentivirus driven by cell specific promoter.
Infectious Antigens	Lentivirus express all kinds of infectious antigens.
Viral Like Particle (VLP)	Lentiviral particles pseudo-typed with high density of surface envelope protein.

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Immuno Therapy	Lentivirus products for Immuno Therapy application.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers
LacZ expression	Express different full length **β- galactosidase (lacZ)** with different selection markers
Anti-miNA lentivirus	Pre-made lentivirus expression a specific **anti-miRNA** cassette.
Pre-made shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more).
microRNA and anti-microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
Negative control lentiviruses	Premade **negative control lentivirus with different markers**: serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme	Ready-to-use lentivirus, expressing **specific enzymes** with different selection markers.