GenTarget Inc
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Pre-made Lentiviral Particles for intracelular labeling:

(LocLight™ Living cell imaging lentivirus for sub-cellular localization)

GenTarget **LocLight™ cell organelle labeling lentivirus** was provided as 200ul/per vial with different fluorescent markers, at the titer of $1x10^7$ IFU/mL. Please see the following table for product information.

LocLight Particles Name	Targeted Sub-cellular Organelle	Product Catalog #		
		GFP	RFP	CFP
Nuc-GFP (Puro), Nuc-RFP (Puro), Nuc-CFP (Puro),	Fluorescent signal only showed in **nucleus area** targeted by an improved **NLS** (Nuclear localization sequence) signal. Particles contains **Puromycin** marker.	LVP360-G & LVP360-G-PBS	LVP360-R & LVP360-R-PBS	LVP360-C & LVP360-C-PBS
Cyto-GFP (Puro), Cyto-RFP (Puro), Cyto-CFP (Puro),	Fluorescent signal only showed in **Cytoplasm area** targeted by an **engineered nuclear export** signal. Particles contains **Puromycin** marker.	LVP450-G	LVP450-R	LVP450-C
ER-GFP (Puro), ER-RFP (Puro), ER-CFP (Puro),	Fluorescent signal targeted in **Endoplasmic Reticulum** (ER) by the C-terminal **KDEL** (ER retention signal). Particles contains **Puromycin** marker.	LVP606-G	LVP606-R	LVP606-C
Golgi-GFP (Bsd), Golgi-RFP (Bsd), Golgi-CFP (Bsd),	Fluorescent signal targeted in **Golgi** by the **golgi retention signal** from 1, 4-galactosyltransferase (GT). Particles contains **Blasticidin** marker.	LVP451-G	LVP451-R	LVP451-C
Mito-GFP (Bsd), Mito-RFP (Bsd), Mito-CFP (Bsd),	Fluorescent signal targeted in **Mitochondria** by the Leader sequence of E1 alpha pyruvate dehydrogenase. Particles contains **Blasticidin** marker.	LVP452-G	LVP452-R	LVP452-C
Mito-GFP (Puro), Mito-RFP (Puro), Mito-CFP (Puro),	Fluorescent signal targeted in **Mitochondria** by the Leader sequence of E1 alpha pyruvate dehydrogenase. Particles contains **Puromycin** marker.	LVP893-G	LVP893-R	LVP893-C
Mito-GFP (Neo), Mito-RFP (Neo), Mito-CFP (Neo),	Fluorescent signal targeted in **Mitochondria** by the Leader sequence of E1 alpha pyruvate dehydrogenase. Particles contains **Neomycin** marker.	LVP894-G	LVP894-R	LVP894-C

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Nuc-membrane-GFP (Puro), Nuc-membrane-RFP (Puro), Nuc-membrane-CFP (Puro),	Fluorescent signal targeted in **Nuclear Membrane** by the inner nuclear membrane localization signal from lamin B membrane receptor. Particles contains **Puromycin** marker.	LVP453-G	LVP453-R	LVP453-C
Peroxisome-GFP (Puro), Peroxisome-RFP (Puro), Peroxisome-CFP (Puro),,	Fluorescent signal targeted in **Peroxisome** by the Peroxisomal C-terminal SKL targeting sequence. Particles contains **Puromycin** marker.	LVP454-G	LVP454-R	LVP454-C
Plasma-mem-GFP (Puro) Plasma-mem-RFP (Puro), Plasma-mem-CFP (Puro),	Fluorescent signal targeted in **Plasma membrane** by ADP-ribosylation factor 6, a plasma membrane protein. Particles contains **Puromycin** marker.	LVP455-G	LVP455-R	LVP455-C
Microtubule-GFP (Puro), Microtubule-RFP (Puro), Microtubule-CFP (Puro),	Fluorescent signal targeted in **Microtubule** by microtubule-associated protein 4 (MAP4). Particles contains **Puromycin** marker.	LVP456-G	LVP456-R	LVP456-C
GFP-H2B (Puro), RFP-H2B (Puro), CFP-H2B (Puro),	Fluorescent signal targeted in **mitotic chromosomes and interphase chromatin** by Histone 2B protein (H2B). Particles contains **Puromycin** marker.	LVP444-G	LVP444-R	LVP444-C
Lysosomes-GFP (Bsd), Lysosomes-RFP (Bsd), Lysosomes-CFP (Bsd),	Fluorescent signal targeted in **Lysosomes** by lysosomal associated membrane protein 1 (LAMP1). Particles contains **Blasticidin** marker.	LVP457-G	LVP457-R	LVP457-C
Endosomes-GFP (Puro), Endosomes-RFP (Puro), Endosomes-CFP (Puro),	Fluorescent signal targeted in **Endosomes** by RAB5A that localized to early endosomes for endocytosis and endocytic-sorting pathways. Particles contains **Puromycin** marker.	LVP458-G	LVP458-R	LVP458-C

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GFP LocLight control, RFP LocLight control, CFP LocLight control,	Fluorescent marker fusioned with a non targeting sequence (Null), serves as controls with fluorescent signal evenly distributed in entire cell. Particles contains **Puromycin** marker.	**Null-G (Puro)**	**Null-R (Puro)**	**Null-C (Puro)**

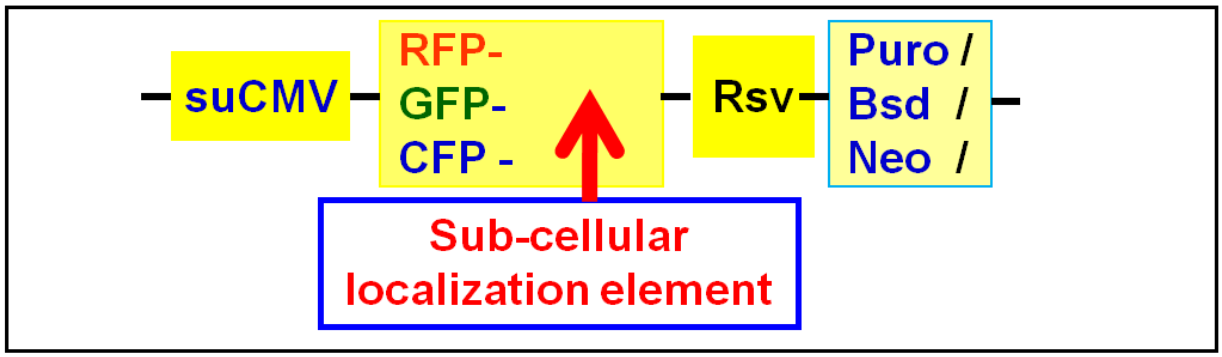
Storage: **<** -70 °C, avoid repeat freeze/thaw cycles. Stable for > 6 months.

Product Description:

Lentivirus is the easiest and most effective method for delivering genes into the majority of mammalian cell types, including non-dividing and primary cells. It allows genes to be integrated into the host cell genome for long-term expression.

Utilizing our proprietary lentiviral vector systems, GenTarget has generated the **LocLight™ Sub-Cellular Labeling Lentivirus** product line. These premade lentiviruses contain a well-defined organelle targeting signal fusioned to a fluorescent protein (GFP, RFP, or CFP) and expressed under our proprietary suCMV promoter. A non-targeting spacer sequence (Null) was fusioned with fluorescent which serves as the control lentivirus. The Null control virus demonstrate a evenly distributed fluorescent signal inside the cell. The lentivirus also contains an antibiotic marker (**Puromycin**, **Neomycin** or **Blasticidin**) under a Rsv promoter (see lentivector's core structure scheme below).

Core expression cassette of LocLight particles:



The fluorescent proteins are non-toxic to cells, do not compromise cell structure, or interfere with signaling pathways. They are therefore ideal tools for live cell imaging and dynamic investigation of sub-cellular signal pathways.

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Pre-made LocLight™ sub-cellular labeling lentiviruses are extremely easy to use, and do not require any additives or substrates. Simply add the lentivirus into a mammalian cell culture. The expression of auto-fluorescent protein will be localized to the specific sub-cellular compartments at 1-3 days post-transduction and can be visualized by fluorescence microscopy. Each particle also contains a puromycin antibiotic selection marker under the control of the RSV promoter (see the vector core structure scheme above) which provides an easy way to select positive cells for long term research.

Key features:

- **Robust Expression and High Titer**: GenTarget's Premade Lentiviral Particles have the brightest fluorescence and the strongest transduction efficiency of any lentiviral particles on the market.
- **Long-Term Expression:** GenTarget's Premade Lentiviral Particles produce long-lasting expression of fluorescently-labeled target proteins even in hard-to-transfect cell lines such as primary and neuronal cells.
- **Easy Transduction:** Simply add the Particles to your cell culture and visualize fluorescence in 48-92 hours. There is no need for any additives or changes of medium.
- **Multiple Colors:** Particles expressing different colors may be transduced into the same cells for **multi-color applications.**
- **Easy Selection of Transduced Cells:** Use either fluorescence signal or puromycin resistance.
- **Tested and Validated:** Each lot of Particles is validated and guaranteed to be of the highest quality.
- **Ready-to-Use:** Simply add virus into your cell culture.

For general questions about our ready-to-use lentiviral particles, please See **FAQs for pre-made lentiviral particles** (.pdf) on our website. (http://www.gentarget.com/wp-content/uploads/2013/07/FAQ-Premade-Lentiviral-particles.pdf).

GenTarget provides **lentiviral services** for cloning your gene of interest and generating ready-to-use viral particles with the best prices and fastest turnaround times. Please see **our website** for details.

Transduction Protocols:

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1. Transduction Protocol for Adhesive cells :

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).

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).

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2. Transduction Protocol for Suspension Cells:

Grow cells in complete suspension culture medium; use a shaking flask in a CO^2 incubator if necessary.

Measure cell density. When density has reached ~3 x 10^6 cells/ml, measured viability should be > 90%. Dilute cells into 1 x 10^6 cell/ml in complete medium.

Day 1:

- Thaw lentiviral particles at room temperature.
- Add premade lentiviral particles into the diluted cells at a ratio of: 50 to 100 µl virus per 0.5 ml of cells (Note: depending on cell type, you may need to use more or less virus).
- Grow cells in a shaking flask in a CO2 incubator.

Day 2:

At 24 hours after transduction, add an equal amount of fresh medium containing relevant antibiotics. **Note:** amount of antibiotic depends on cell type. Continue growing cells in CO2 incubator.

Day 3:

At 72 hours after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

Transduction example images:

50 ul of each particles was added into HEK293 cells (in one well at 24-well plate). Image was taken at 48 hours after virus addition.

Note: Filter wavelength settings:

CFP filter: ~Ex436 ~Em480; GFP filter: ~Ex450-490 ~Em525;
RFP filter: ~Ex545 ~Em620; BFP filter: ~Ex380 ~Em460;

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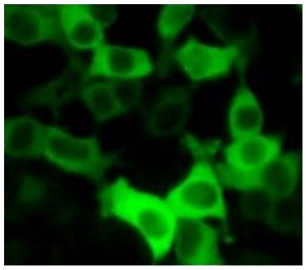
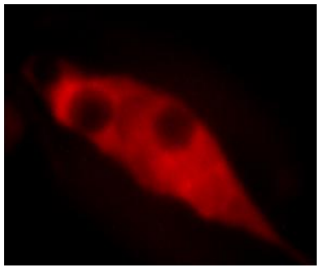
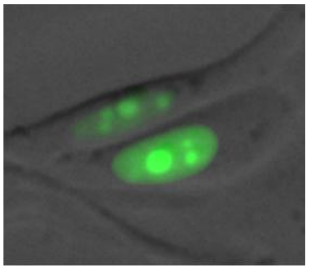


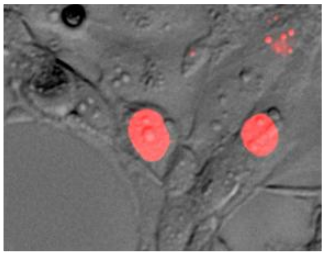
Cyto-GFP
(CAT# **LVP450-G**)



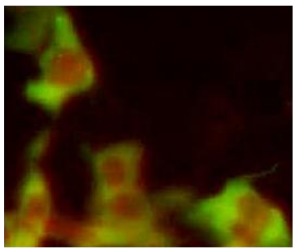
Cyto-RFP
(CAT# **LVP450-R**)



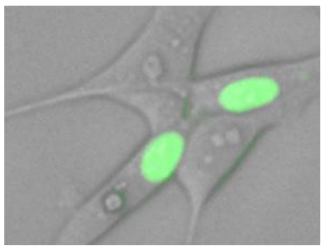
Nuc-GFP
(CAT# **LVP360-G**)



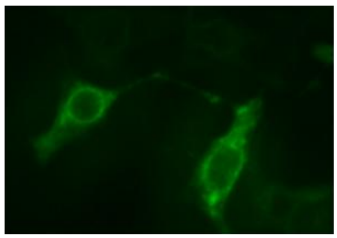
Nuc-RFP
(CAT# **LVP360-R**)



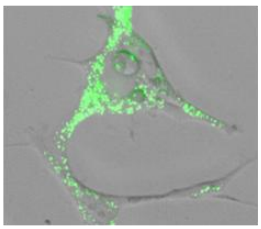
Cyto-GFP + Nuc-RFP
(CAT# **LVP450-G + LVP360-R**)



GFP-H2B,
(CAT# **LVP440-G**)



Nuc-membrane-GFP,
(CAT# **LVP453-G**)



Peroxisome-GFP
(CAT# **LVP454-G**)

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

References:

1. J Virol. 2000 November; 74(22): 10778–10784.
2. Hum Gene Ther (2003) 14: 1089-105.
3. Mol Ther (2002) 6: 162-8.
4. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors. (Link).

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.

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Related Products:

Product Category	Product Description (please click category name to see product's pages)
Human and mouse ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP**.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla** with different antibiotic selection markers.
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.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing with **CRISPR**
TetR inducible expression repressor	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
Signal Pathway monitoring	Premade lentivirus for monitoring each signal transductin pathway activiviy and for making your desired signal pathway report cell lines.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers
T-antigen Expression	Express different large and small T antigen with different selection markers
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent marker **GFP/RFP/CFP was sub-cellular localized** in different cell organelle for living cell imaging.
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.
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
Pre-made shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more).
microRNA	Premade lentivirus expression human or mouse **precursor**

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and anti-microRNA lentivirus	**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 expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.