GenTarget Inc

7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Fax: 1 (800) 380-4198
Email: orders@gentarget.com

Pre-made Lentiviral Particles for intracelular labeling:

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

Product Name	Produce Description	Product Catalog #
Nuc-GFP (Puro), **Nuc-RFP (Puro),** **Nuc-CFP (Puro),** **Nuc-GFP (Neo),** **Nuc-RFP (Neo),** **Nuc-RFP (Bsd),** **Nuc-BFP (Puro)**	**Nucleus** localized different fluorescent marker, with different antibiotic selection	LVP360-G; LVP360-G-PBS LVP360-R; LVP360-R-PBS LVP360-C; LVP360-C-PBS LVP360-GN LVP360-RN LVP360-RB LVP1495
Cyto-GFP (Puro), **Cyto-RFP (Puro),** **Cyto-CFP (Puro),** **Cyto-BFP (Puro),**	**Cytoplasm area** targeted fluorescent marker	LVP450-G LVP450-R LVP450-C LVP1496
ER-GFP (Puro), **ER-RFP (Puro),** **ER-CFP (Puro),** **ER-BFP (Puro),**	**Endoplasmic Reticulum** (ER) targeted fluorescent marker, by the C-terminal **KDEL** (ER retention signal).	LVP606-G LVP606-R LVP606-C LVP1497
Golgi-GFP (Bsd), **Golgi-RFP (Bsd),** **Golgi-CFP (Bsd),** **Golgi-BFP (Puro),**	**Golgi** targeted fluorescent marker, by the **golgi retention signal** from 1, 4-galactosyltransferase (GT).	LVP451-G LVP451-R LVP451-C LVP1498
Mito-GFP (Bsd), **Mito-RFP (Bsd),** **Mito-CFP (Bsd),** **Mito-GFP (Puro),** **Mito-RFP (Puro),** **Mito-CFP (Puro),** **Mito-GFP (Neo),** **Mito-RFP (Neo),** **Mito-CFP (Neo),** **Mito-BFP (Puro),**	**Mitochondria** targeted fluorescent marker, by the Leader sequence of E1 alpha pyruvate dehydrogenase.	LVP452-G LVP452-R LVP452-C LVP893-G LVP893-R LVP893-C LVP894-G LVP894-R LVP894-C LVP1499
Nuc-membrane-GFP (Puro), **Nuc-membrane-RFP (Puro),** **Nuc-membrane-CFP (Puro),** **Nuc-membrane-BFP (Puro),**	**Nuclear Membrane** targeted fluorescent marker, by the inner nuclear membrane localization signal from lamin B membrane receptor.	LVP453-G-PBS LVP453-R LVP453-C LVP1500

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Peroxisome-GFP (Puro), **Peroxisome-RFP (Puro),** **Peroxisome-CFP (Puro),** **Peroxisome-BFP (Puro),**	**Peroxisome** targeted fluorescent marker, by the Peroxisomal C-terminal SKL targeting sequence.	LVP454-G LVP454-R LVP454-C LVP1501
Plasma-mem-GFP (Puro) **Plasma-mem-RFP (Puro),** **Plasma-mem-CFP (Puro),** **Plasma-mem-BFP (Puro),**	**Plasma membrane** targeted fluorescent marker , by ADP-ribosylation factor 6, a plasma membrane protein.	LVP455-G LVP455-R LVP455-C
Microtubule-GFP (Puro), **Microtubule-RFP (Puro),** **Microtubule-CFP (Puro),** **Microtubule-BFP (Puro),**	**Microtubule** targeted fluorescent marker, by microtubule-associated protein 4 (MAP4).	LVP456-G LVP456-R LVP456-C LVP1503
GFP-H2B (Puro), **RFP-H2B (Puro),** **CFP-H2B (Puro),** **BFP-H2B (Puro),**	**mitotic chromosomes and interphase chromatin** targeted fluorescent marker, by Histone 2B protein (H2B).	LVP444-G LVP444-R LVP444-C LVP1492
Lysosomes-GFP (Bsd), **Lysosomes-RFP (Bsd),** **Lysosomes-CFP (Bsd),** **Lysosomes-BFP (Puro),**	**Lysosomes** targeted fluorescent marker, by lysosomal associated membrane protein 1 (LAMP1).	LVP457-G LVP457-R LVP457-C
Endosomes-GFP (Puro), **Endosomes-RFP (Puro),** **Endosomes-CFP (Puro),** **Endosomes-BFP (Puro),**	**Endosomes** targeted fluorescent marker, by RAB5A that localized to early endosomes for endocytosis and endocytic-sorting pathways.	LVP458-G LVP458-R LVP458-C LVP1505
GFP LocLight control, **RFP LocLight control,** **CFP LocLight control,** **BFP LocLight control,**	Fluorescent marker fusioned with a non targeting sequence (Null), serves the non-targeted fluorescent signal controls.	Null-G (Puro) Null-R (Puro) Null-C (Puro) Null-B (Puro)

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

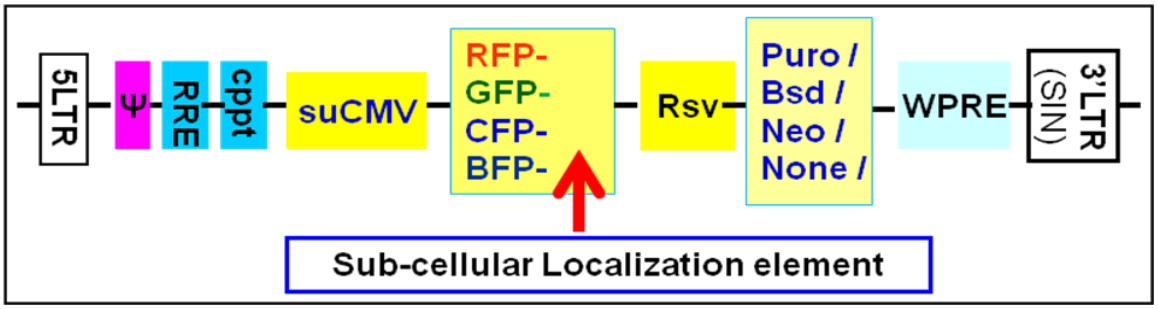
Product Description:

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**, **CFP** or **BFP**) 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, or no any antibiotic selection (**None**) (see lentivector's core structure scheme below).

Scheme of "LocaLight" Lentivectors:



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.

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 antibiotic selection which provides the selection for the transducd cells if desired. Or you can simply sort the transduced via its Fluorescent signal.

Key features:

- **Robust and High Titer**: GenTarget's Premade Lentiviral Particles have the brightest fluorescence and the strongest transduction efficiency of any lentiviral particles on the market. Lentivirus are provided in two formats, crude lentivirus; and concentrated lentivirus in PBS which is best used for suspension cells, or "hard-to-transduced" cell types
- **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.

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- **Easy Transduction:** All premixed with 10x Polybrene (60ug/ml). Simply add the Particles to your cell culture, wait 48-92 hours, then visualize fluorescence. There is no need for any additives or medium changes.
- **Multiple Colors:** Particles expressing different colors may be transduced into the same cells for **multi-color applications.**
- **Easy Selection of the transduced Cells:** Use either fluorescence signal or antibiotic killing selection.
- **Tested and Validated:** Each lot of Particles is validated and guaranteed to be of the highest quality.

For general questions about our ready-to-use lentiviral particles, please See *FAQs for pre-made lentiviral particles* (.pdf) on our website.

Transduction Protocols:

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 or just 50ul lentivirus/per well in 24w/p.

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- 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).

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. 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 24~48 hours post virus addition, 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.

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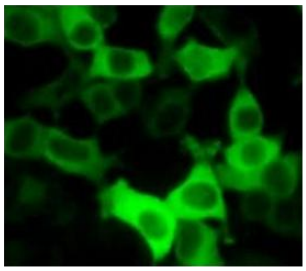
Transduction example images:

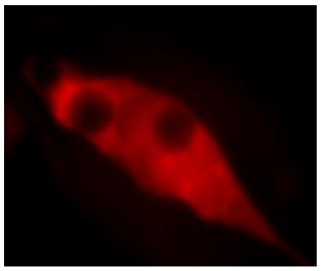
50 ul of each particles was added into HEK293 cells (in one well at 24-well plate). Image was taken at 48 hours after virus addition.

Note: Filter wavelength settings:

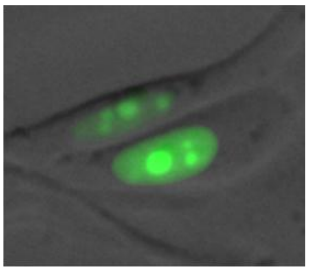
CFP filter: ~Ex436 ~Em480; GFP filter: ~Ex450-490 ~Em525;
RFP filter: ~Ex550 ~Em583; BFP filter: ~Ex380 ~Em460;



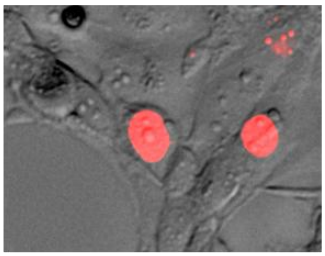
Cyto-GFP
(CAT# **LVP450-G**)



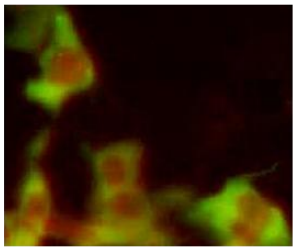
Cyto-RFP
(CAT# **LVP450-R**)



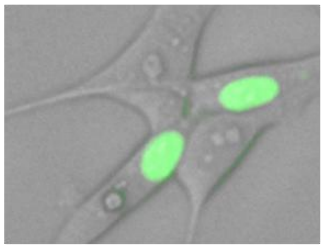
Nuc-GFP
(CAT# **LVP360-G**)



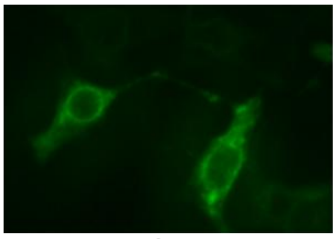
Nuc-RFP
(CAT# **LVP360-R**)



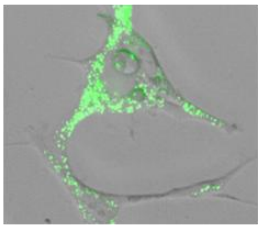
Cyto-GFP + Nuc-RFP
(CAT# **LVP450-G + LVP360-R**)



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:

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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	Repoter 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, 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/ TRαV3-F2A-TRβV5-6;
CRISPR Gene Editing	Preamde lentivirus express humanzied 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	Llentivirus 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.

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Non-integrating LV	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
shRNA Knockdown	Knockdown verifeid 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-miNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
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 fusioned 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.

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Product Category	Product Description (please click into each category's page)
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.
CRE, Flp ColorSwtich	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.
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	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
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.
Ultra titer lentivirus	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.