GenTarget Inc

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

Lentiviral Particles for Fluorescent Labelled Cell Skeleton Protein

Catalog#	Product Name	Amounts
LVP446-G	**GFP-Actin (Puro)** Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP446-R	**RFP-Actin (Puro)** Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP446-C	**CFP-Actin (Puro)** Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP1338-G	**GFP-Tubulin (Puro)** Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP1338-R	**RFP-Tubulin (Puro)** Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP1338-C	**CFP-Tubulin (Puro)** Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP1339-G	**GFP-Paxillin (Puro)** Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP1339-R	**RFP-Paxillin (Puro)** Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP1339-C	**CFP-Paxillin (Puro)** Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP1340-G	**GFP-Vimentin (Puro)** Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP1340-R	**RFP-Vimentin (Puro)** Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP1340-C	**CFP-Vimentin (Puro)** Lentiviral particles	$1x10^7$ IFU/ml x 200ul

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

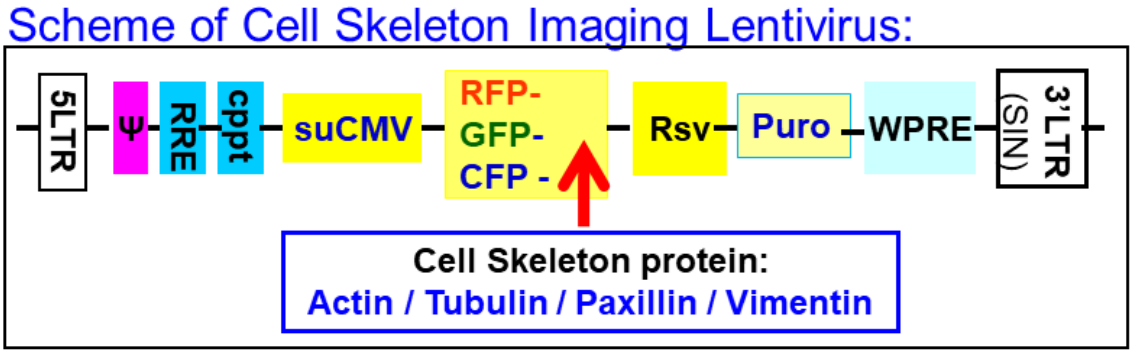
Product Introduction:

GenTarget's Lentiviral gene delivery system uses Human Immunodeficiency Virus-1 (HIV) lentivector plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into virtually 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 lentivirus a great gene transfer agent.

The lentivirus for the Cell Skeleton structure imaging were constructed by incorporated a fluorescent marker (**GFP-, RFP-, CFP-**) at N-terminal of a cell skeleton structure protein (**β-actin, α-tubulin, paxillin, vimentin)** as a fusion target. Under Fluorescent-microscope, the cytoskeletal structure demonstrated fluorescent signal. It provides a convenient method for visualization of cytoskeletal structure and dynamics in real-time.

GenTarget Inc
7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: (858) 2656446
Fax: (800) 3804198
Orders@gentarget.com

The Fluorescent-target fusion constructs are expressed under an enhanced CMV promoter. The lentivirus also carries the **Puromycin** antibiotic selection under Rsv promoter. See vector scheme below for vector core structure.



1) **Actin**
Actins are highly conserved proteins that are involved in cell motility, structure, and integrity. Three main groups of actin isoforms, alpha, beta, and gamma, constituent of the contractile apparatus. The β-actin is the primary component of the cytoskeleton. Fluorescent labelled Actin provides a convenient tool to invest the dynamic mechanisms of stress fiber assembly and adaptation to strain.

2) Tubulin
Microtubules are dynamic cytoskeletal filaments composed of α- and β-tubulins. Microtubules function in many essential cellular processes. The fluorescent tagged tubulin can be used to image microtubule dynamics, providing the insight to our understanding of microtubule-binding agents.

3) Paxillin (PAX)
Paxillin is expressed at focal adhesions of non-striated cells and at costameres of striated muscle cells, and it functions to adhere cells to the extracellular matrix. Paxillin is a signal transduction adaptor protein.

The fluorescent labelled paxillin provide a tool to analyze focal adhesion dynamics in live cells. Focal adhesions are highly dynamic structures that assemble at the leading edge of the migrating cell and disassemble at the trailing edge.

4) Vimentin (VIM)
Vimentin (VIM) is a type III intermediate filament (IF). IF, tubulin-based microtubules and actin-based microfilaments, comprises the cytoskeleton. vimentin is the major cytoskeletal component of mesenchymal cells.

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

Vimentin is often used as a marker of cells undergoing an epithelial-to-mesenchymal transition (EMT) during both normal development and metastatic progression. The fluorescent labelled Vimentin provide insight on the dynamics of vimentin. When cells change from Mesenchymal To Epithelial (MET), the VIM-tagged fluorescent signal will be reduced correspondingly, which serves the real-time monitoring the cell status changes.

Product application:

These Lentiviral Particles are great tools for:

- Sub-cellular live cell visualization;
- *in vivo* signal transduction research;
- Live cell imaging, protein interaction studies and many other applications;

Product Features:

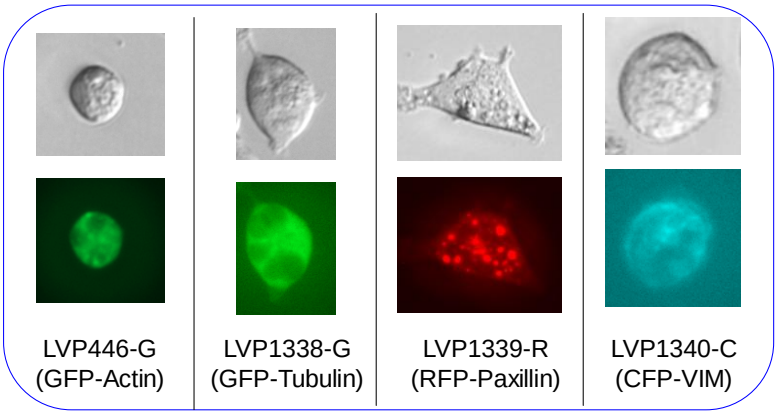
- **Robust Expression and High Titer**: GenTarget's Premade Lentiviral Particles have the brightest fluorescence signal driven by the strongest promoter suCMV;
- **Long-Term Expression:** The transduced cells can be selected by Puromycin selection for generation of stable cell line for long term study.
- **Easy Transduction and easy, ready to use:** 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:** Lentivirus contain different colors, convenient for multi-color applications for combined labeling.
- **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.

If you would like GenTarget to make lentiviral particles expressing a specific target-fluorescent protein fusion, we can do so as a custom lentiviral services. We will clone your gene of interest and generate ready-to-use viral particles. Our prices are the best, our turnaround times are the fastest in the industry. Please contact us for details.

GenTarget Inc
7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: (858) 2656446
Fax: (800) 3804198
Orders@gentarget.com

Sample images of Cell Skeleton lentivirus:



Add 50ul each lentivirus into one well in 24-well-plate where cell density is at 50% ~ 75% in different cell types (HEK293 cells). Image taken under Fluorescent-Microscope at ~72 hours after lentivirus added.

Note: Filter wavelength settings:
GFP filter: ~Ex450-490 ~Em525;
RFP filter: ~Ex545 ~Em620;
CFP filter: ~Ex436 ~Em480;

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:

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

- 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. Do nothing.
  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 48hr~72hr (Depend upon cell type) 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, or select by antibiotic killing. 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 required.

Measure cell density (not grow over 3 million/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. Continue growing cells in CO2 incubator.

Day 3+:

At 48 hour to 72 hours (Depend upon cell type) after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Pass cells into 0.5 million/ml density in completed medium containing the

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

corresponding antibiotic (**Note:** amount of antibiotic depends on cell type. A killing curve must pre-established). Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

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. 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.

Attachment: GenTarget's pre-made lentivirus product categories.

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Lentivirus for all kinds of pathway 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-T, TCR-T, Assay cell lines, and Cell Antigens & Receptors.
CRISPR Gene Editing	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease, the **dCas9**, gRNAs, **CRISPR** gene editing research

GenTarget Inc
7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: (858) 2656446
Fax: (800) 3804198
Orders@gentarget.com

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.
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
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

GenTarget Inc

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

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.
LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE 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.