GenTarget Inc

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Pre-made Lentiviral Particles for Fluorescent-Target fusion proteins

Catalog#	Product Name	Amounts
LVP673	**GFP-Luciferase** (**Puro**) fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP674	**RFP-Luciferase** (**Puro**) fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP675	**CFP-Luciferase** (**Puro**) fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP676	**GFP-Luciferase** (**Neo**) fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP677	**RFP -Luciferase** (**Neo**) fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP906	**CFP-Luciferase** (**Neo**) fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP442	**GFP-RFP (Puro)** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP443	**CFP-RFP (Puro)** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP399-R	**RFP-LC3 (Puro)** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP399-G	**GFP -LC3 (Puro)** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP399-C	**CFP -LC3 (Puro)** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP444-G	**GFP-Histone2B (Puro)** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP444-R	**RFP-Histone2B (Puro)** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP444-RB	**RFP-Histone2B (Bsd)** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP444-C	**CFP-Histone2B (Puro)** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP445-G	**GFP-Annexin5 (Puro)** fusion Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP445-R	**RFP-Annexin5 (Puro)** fusion Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP445-C	**CFP-Annexin5 (Puro)** fusion Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP446-G	**GFP-Actin (Puro)** fusion Lentiviral particles	$1x10^7$ IFU/ml x 200ul

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LVP446-R	**RFP-Actin (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP446-C	**CFP-Actin (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP447-G	**GFP-TAT (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP447-R	**RFP-TAT (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP447-C	**CFP-TAT (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP448-G	**GFP-hP53 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP448-R	**RFP-hP53 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP448-C	**CFP-hP53 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP449-G	**GFP-Zyxin (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP449-R	**RFP-Zyxin (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP449-C	**CFP-Zyxin (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP550-R	**RFP-CLCN2 (Puro)** fusion entiviral particles	1x10^7 IFU/ml x 200ul
LVP551-R	**RFP-KCNN4 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP552-R	**RFP-TRPV1 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP554-R	**RFP-TRPC3 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP556-R	**RFP-CSF1 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP556-G	**GFP -CSF1 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP550-C	**CFP-CLCN2 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP551-C	**CFP-KCNN4 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP552-C	**CFP-TRPV1 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP554-C	**CFP-TRPC3 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul
LVP556-C	**CFP-CSF1 (Puro)** fusion Lentiviral particles	1x10^7 IFU/ml x 200ul

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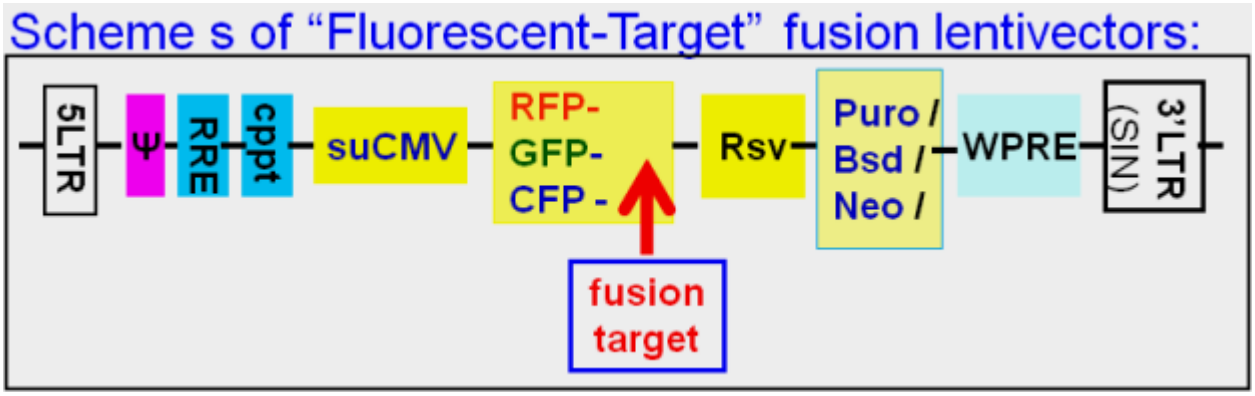
Null-G	**GFP-**Null **(Puro)** fusion control	1x10^7 IFU/ml x 200ul
Null-R	**RFP-**Null **(Puro)** fusion control	1x10^7 IFU/ml x 200ul
Null-C	**CFP-**Null **(Puro)** fusion control	1x10^7 IFU/ml x 200ul

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

Product Description:

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.

Pre-made lentiviral particles, expressing **"GFP-, RFP-, CFP-Target"** fusion constructs, are generated from GenTarget's high expression lentiviral system. A fluorescent protein, GFP, RFP, or CFP is cloned in frame with a target such as human or mouse ORF, and expressed under a proprietary **suCMV promoter** that demonstrates the highest expression level (3-10 folds higher than the CMV promoter in pCDNA6.3 vector, depending upon cell type). Each fluorescent protein was codon optimized to generate a brighter fluorescent-fusion signal. A non-sense sequence (200bp), the "Null control sequence" was cloned in the same lentivector that serves as "Fluorescent-Null" fusion controls. The vector also contains an antibiotic marker (**Puromycin, or Blasticidin or Neomycin dependent each product**) under Rsv promoter. See vector scheme below for vector core structure.



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These Lentiviral Particles are great tools for:

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

The positively transduced cells can be sorted by the fluorescent signal or selected for puromycin / Neomycin / Blasticidin resistance dependent on the product marker selection type.

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 fluorescent 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 the cell culture.

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 and our turnaround times are the fastest in the industry. Please contact us 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).

2) Transduction Protocol for Suspension Cells:

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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.

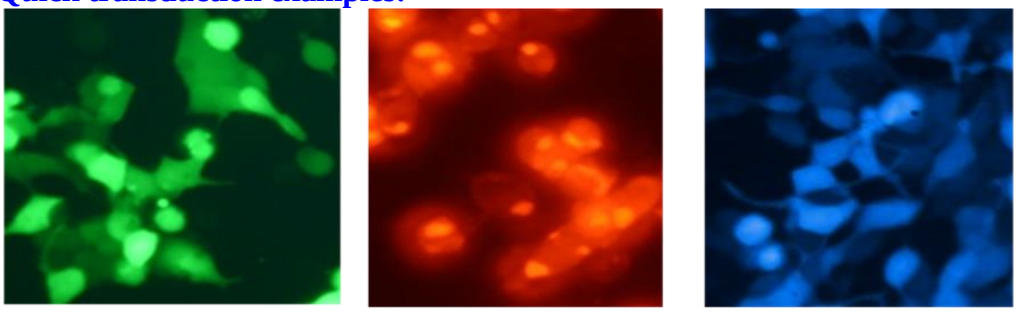
Day 2:

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.

Quick transduction examples:



LVP442 (50ul) (GFP filter) | LVP447-R (50ul) RFP filter | LVP448-C (50ul) CFP filter

Add 50ul each lentivirus into one well in 24-well-plate where cell density is at 50% ~ 75% in different cell types (HEK293, A549, PC3 from left to right). Image taken at ~72 hours after virus added (no medium changed). **Result:** The positive >90%.

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Note: Filter wavelength settings:

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

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.

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Attachment: GenTarget's Pre-made lentivirus 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	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.

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repressor	
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.
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 expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.