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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
LVP442	GFP-RFP fusion **control** lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP443	CFP-RFP fusion **control** lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP399-R	RFP-**LC3** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP399-G	GFP -**LC3** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP399-C	CFP -**LC3** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP444-G	GFP-**Histone 2B** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP444-R	RFP-**Histone 2B** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP444-C	CFP-**Histone 2B** fusion lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP445-G	GFP-**Annexin5** fusion Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP445-R	RFP-**Annexin5** fusion Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP445-C	CFP-**Annexin5** fusion Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP446-G	GFP-**Actin** fusion Lentiviral particles	$1x10^7$ IFU/ml x 200ul
LVP446-R	RFP-**Actin** fusion Lentiviral particles	$1x10^7$ IFU/ml x 200ul

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

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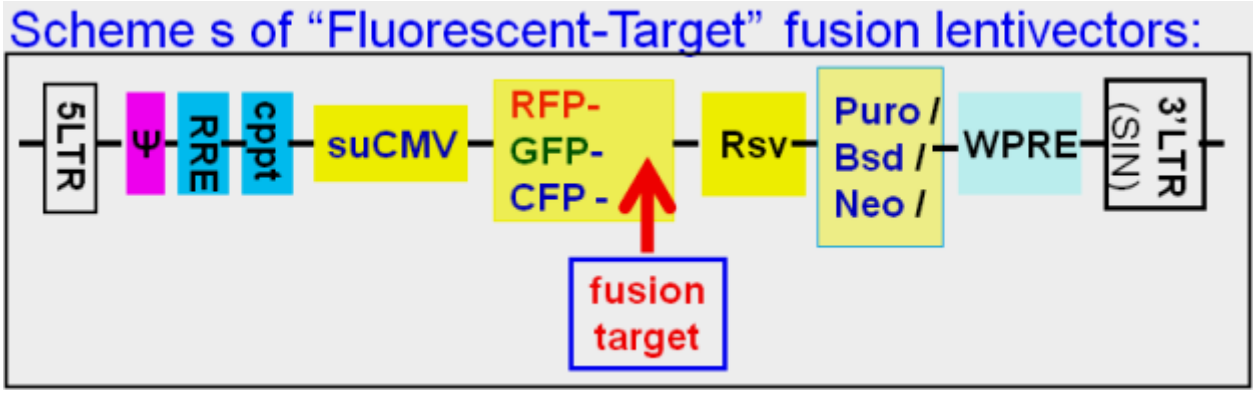
Null-R	RFP-Null fusion control	$1x10^7$ IFU/ml x 200ul
Null-C	CFP-Null fusion control	$1x10^7$ IFU/ml x 200ul

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

Product Description:

Lentiviral system is a gene delivery tool that uses lentivectors for gene expression or knockdown. Lentivectors are HIV-1 (Human Immunodeficiency Virus 1) derived plasmids, used to generate lentiviral particles (lentivirus) that can be transduced into all kind of mammalian cell types or organs, including stem cells, primary cells and non-dividing cells both ***in vivo*** and in **cell culture** system. Particles stably integrate into the transduced cell's genome for long term expression. Therefore, lentivirus holds unique promise as a gene transfer agent.

Pre-made lentiviral particles, expressing **"GFP/RFP/CFP-Target"** fusion constructs, are generated from Amsbio'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), expressed under a proprietary **suCMV promoter** that demonstrates the highest expression levels (3-10 fold higher than CMV promoter in pCDNA6.3 vector - cell type dependent). Each fluorescent protein is codon optimized to generate brighter fluorescent signal. **They are great tools for:** sub-cellular pathway studies, in vivo signal transduction research, living cell imaging, protein interaction studies and many other applications. The positive transduced cells can be sorted via the fluorescent signal or via Puromycin antibiotic selection. (See vector scheme below for vector structures).



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These Lentiviral Particles are great tool 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 depending on the product marker selection type.

Key features:

1. **High titer and robust**: Amsbio's premade lentiviral particles are best in the class, demonstrating the brightest fluorescent signal and strong transduction efficiency. All particles are validated on lot by lot basis and their quality is guaranteed.
2. Delivery of fluorescent labeled targets into hard to transfect cell lines (like primary cells or neuron cells) for **long term expression;**
3. **Very easy to use:** Particles are provided in DMEM medium with 10% FBS and 60ug/ml polybrene for ready to use status. Simply add into your cell culture and visualize the fluorescence after 48-92 hours (No need for any additives, components or medium changes);
4. Different fluorescent labeled particles for **multi-color application** when using multiple different particles in same cells;
5. **Easy selection** via Fluorescent signal or antibiotic marker.

Transduction Protocols:

1) Transduction Protocol for Adhesive cells :

Note: Pre-made lentivirus is provided ready to use, so it can be simply added to your cell culture; the amount of virus to add depends on the 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, passage 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 freeze/thaw cycles. If you do not use all of the virus at once, 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 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. When density has reached ~3 x 10^6 cells/ml, measure 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 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 CO_2 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 CO_2 incubator.

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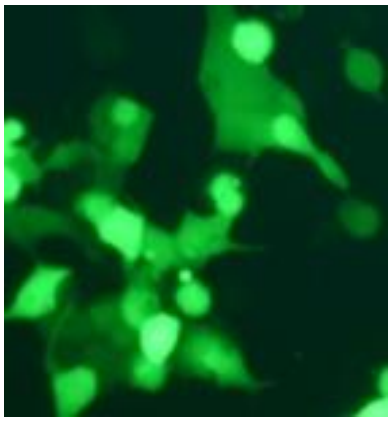
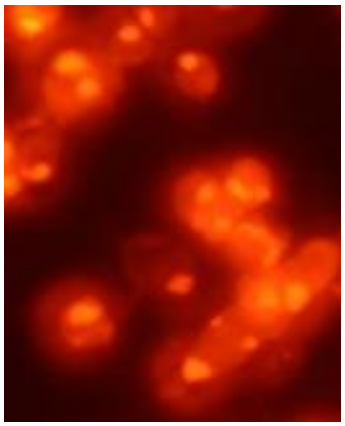
Day 3:

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.

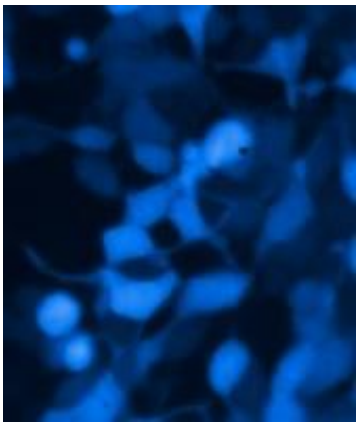
Quick transduction examples:



LVP442 (50ul)
(GFP filter)



LVP447-R
RFP filter



LVP448-C
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). Images taken ~72 hours after virus added (no medium changed). **Result:** >90% of positive transduced cells.

Note: Filter wavelength settings:

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

Attachment: Pre-made lentivirus products:

Product Category	Product Description (please Category name to see product's pages)
Fluorescent protein	Premade Lentivirus for GFP/ CFP/ YFP/ RFP
Human and mouse ORF	Premade lentivirus expressing **human and mouse ORFs** with RFP-Blasticidin fusion dual markers.

Luciferase expression	Premade lentivirus for 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.
TetR inducible expression repressor	Premade lentivirus expressing TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
iPS factors	Premade lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibiotic 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** is localized in different cell organelles for living cell imaging.
LacZ expression	Express full length **β- galactosidase (lacZ)** with different selection markers
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 negative control for lentivurs treatment, for validation of the specificity of lentivirus target expression effect.
Other Enzyme expression	Ready-to-use lentivirus, expressing a specific enzyme with different selection markers.

Safety Precaution:

Amsbio lentiviral particles have adopted the most 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 gloves at all times when handling lentiviral particles! Please refer to CDC and NIH's guidelines for more details regarding the 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
5. CDC guidelines for Lab Biosafety levels link

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Warranty:

This product is for research use only. It is warranted to meet its quality as described when used accordance with its instructions. Amsbio disclaims any implied warranty of this product for particular application. In no event shall Amsbio be liable for any incidental or consequential damages in connection with the products. Amsbio's sole remedy for breach of this warranty should be, at Amsbio's option, to replace the products.

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