



## Pre-made Lentiviral Particles for Fluorescent Proteins

Catalog#	Product Name	Amounts
<b>Fluorescent proteins expressed under suCMV promoter:</b>		
LVP001	<b>GFP (Bsd)</b> lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP001-PBS	<b>GFP (Bsd)</b> lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP002	<b>GFP-His (bicistronical RFP)</b> lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP002-PBS	<b>GFP-His (bicistronical RFP)</b> lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP011	<b>CFP (Bsd)</b> lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP011-PBS	<b>CFP (Bsd)</b> lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP012	<b>YFP (Bsd)</b> lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP012-PBS	<b>YFP (Bsd)</b> lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP023	<b>RFP (Bsd)</b> Lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP023-PBS	<b>RFP (Bsd)</b> lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP299	<b>RFP (Neo)</b> lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP299-PBS	<b>RFP (Neo)</b> lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP300	<b>GFP (Neo)</b> lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP300-PBS	<b>GFP (Neo)</b> lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP306	<b>CFP (Neo)</b> lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP306-PBS	<b>CFP (Neo)</b> lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP307	<b>YFP (Neo)</b> lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP307-PBS	<b>YFP (Neo)</b> lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP309	<b>RFP (Puro)</b> lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP309-PBS	<b>RFP (Puro)</b> lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP340	<b>GFP (puro)</b> lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP340-PBS	<b>GFP (Puro)</b> lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP342	<b>CFP (puro)</b> lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP342-PBS	<b>CFP (Puro)</b> lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul



Catalog#	Product Name	Amounts
<b>Fluorescent proteins expressed under EF1a promoter:</b>		
LVP310	GFP (EF1a)-Bsd lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP310-PBS	GFP (EF1a)-Bsd lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP425	GFP (EF1a)-Neo lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP425-PBS	GFP (EF1a)-Neo lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP426	GFP (EF1a)-Puro lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP426-PBS	GFP (EF1a)-Puro lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP427	RFP (EF1a)-Bsd lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP427-PBS	RFP (EF1a)-Bsd lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP428	RFP (EF1a)-Neo lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP428-PBS	RFP (EF1a)-Neo lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP429	RFP (EF1a)-Puro lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP429-PBS	RFP (EF1a)-Puro lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP430	CFP (EF1a)-Bsd lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP430-PBS	CFP (EF1a)-Bsd lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP431	CFP (EF1a)-Neo lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP431-PBS	CFP (EF1a)-Neo lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul
LVP432	CFP (EF1a)-Puro lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
LVP432-PBS	CFP (EF1a)-Puro lentiviral particles, in vivo ready	1x10 <sup>8</sup> IFU/ml x 200ul

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

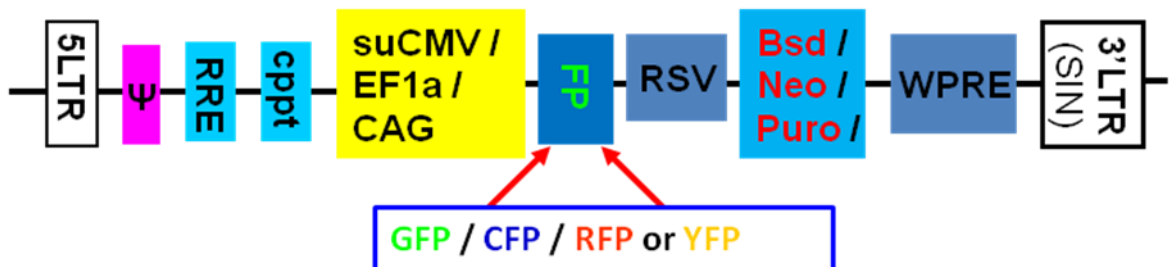


## Product Description:

Lentiviral system is a gene delivery tool using 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 virtually all kinds 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 cells' genome for long term expression. Therefore, lentivirus holds unique promise as gene transfer agents.

Pre-made lentiviral particles expressing **fluorescent proteins** are generated from GenTarget's **high expression lentiviral system** with different promoters. The proprietary **suCMV promoter** demonstrated the highest expression levels in majority cell types. The **engineered EF1a promoter** is non-tissue specific, highly expressed in all cell types, and less likely be silenced after long-term culture. The **CAG promoter** ( a hybrid of the CMV enhancer and chicken beta-actin promoter) has the highest expression level in embryonic stem cells. Fluorescent gene was cloned into lentivectors with **different selection antibiotic markers** (See vector scheme below for vector structures). Each fluorescent was codon optimized to generate brighter fluorescent signal. Then, expression lentivectors were co-transfected with Gentarget's proprietary packaging mix (Cat# [HT-pack](#)) into a 293T cell (cat# [TLV-C](#)). The pre-made lentiviral particles are VSV-G pseudotyped viruses. The fluorescent proteins are expressed under three different constitutive promoters to meet different needs.

### Schematic representation of lentivector for FP controls



### Bicistronic vector (Cat#: LVP020)





Gentarget's premade lentiviral particles are best in the class, demonstrating the brightest fluorescent signal, strong transduction efficiency. Each virus was validated in lot by lot basis and its quality is guaranteed.

### Particles were provided in two formats:

1. Regular particles in DMEM medium with 10% FBS and 60ug/ml polybrene;
2. Particles concentrated and buffer exchanged into PBS as *in vivo ready* status.

For general questions about our ready-to-use lentiviral particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf) on our website.  
(<http://www.gentarget.com/pdf/FAQ-Premade-Lentiviral-particles.pdf>).

Gentarget Inc also provides lentiviral services for cloning your gene of interest and generates ready-to-use viral particles with the best prices and fastest turnaround time. Please see [our website](#) for details.

## Transduction Protocols:

### 1. Adhesive cells Transduction Protocols:

**Note: A quick transduction protocol is: add 50ul virus into one well in 24-well-plate where cell density is at 50% ~ 75%. At 72 hours after virus added (no need to change medium), visualize the positive rate under fluorescent microscope. For stable cell line generation, pass cell into antibiotic containing medium, or sort the cells via fluorescent signal. Then , select the cells by antibiotics.**

**Day 0:** Seed the desired cells in complete medium at appropriate density incubate overnight. (Note: at the time of transduction, it grows to 50% ~75% confluent.)  
For example, seed Hela cells at  $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$  in a well of a 24-well plate;

**Day 1:** Remove the culture medium. Add fresh, warmed, complete medium (0.5ml). Thaw the Pre-made lentiviral stock at room temperature. Add appropriate amount of virus stock to obtain the desired MOI. Return cells to 37°C/CO<sup>2</sup> incubator. (Try to avoid thaw and freeze cycles for pre-made lentivirus. But if you cannot use all virus in one time, you still can re-freeze the virus at -80oC for future use. But virus titer will decrease by ~10% for each re-thaw.)

**Day 3:** At ~72hr after transduction, check the transduction rate *via* fluorescence image with a suitable filter under fluorescent microscope, or calculate the exact transduction rate via Flow Cytometry System (FACS) or any flow cytometry (such as Guava machine).



**Day 3 +** (optional): Transduced cells can be sorted out via FACS, selected by its specific antibiotics. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line. (Refer to any literatures about How to generate stable cell lines.)

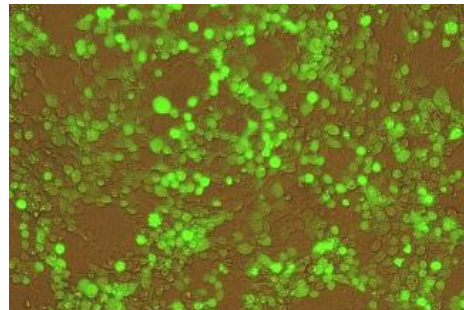
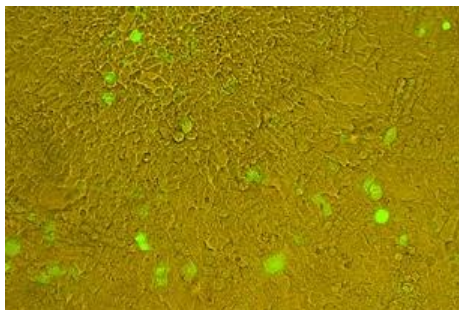
## 2. Suspension cells transduction Protocols:

1. Grow your cell in your completed suspension culture medium, shaking in flask in CO<sup>2</sup> incubator if necessary;
2. Measure cell density. When cell grow to  $\sim 3 \times 10^6$  cell/ml, measure cell viability (should be > 90%), then diluted cells into  $1 \times 10^6$  cell/ml in completed medium;
3. Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particle into the diluted cells at ratio of: **50 to 100ul virus per 0.5 ml of cells** (Note: depending on the cell types; you may need to use more or less viruses). Grow cells in flask, shaking in CO<sub>2</sub> incubator.
4. At 24 hours after transduction, add equal amount of fresh medium containing related antibiotics (Note: each particles contain an antibiotic marker and the antibiotic amounts to use depends upon cell types). Grow cell in CO<sub>2</sub> incubator.
5. At 72 hours after transduction, check fluorescence under microscope or calculate the transduction efficiency using cell sorting machine (like FACS or Guava machine).
6. You can sort the fluorescent positive cells, and maintain the antibiotic selection to generate stable cell lines.

(Note: GFP filter wavelength: Ex450-490 ~Em525; RFP filter: ~Ex545/~Em620. Fusion marker has slightly shifted wavelength, but no need for filter changes.)

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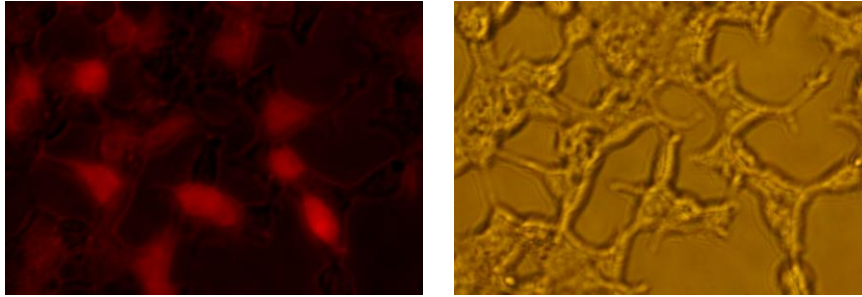
### Transduction Example A:



**Figure 1: GFP Expression in HeLa cells.** HeLa cells were transduced with 5ul (Right image) or 50ul (Left image) of Pre-made GFP lentivirus (#LVP001) in 24-well plate (see protocol above). GFP signal was visualized at 72 hours after transduction.



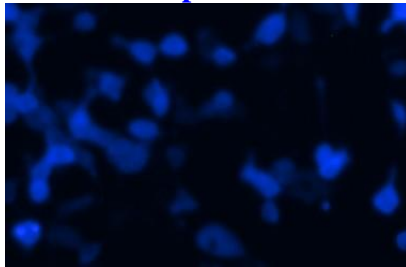
### Transduction Example B:



**Figure 2: RFP Expression in A549 cells.** A549 cells were transduced with 50ul Pre-made RFP lentivirus (Cat#: LVP023-SF) in 24-well plate. RFP signal was visualized at 72 hours after transduction. Images were taken under microscope, the left image under RFP filter, the right image under bright light.

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### Transduction Example C:



**Figure 3: CFP Expression in HEK293 cells: Quick transduction protocol:** add 50ul CFP lentivirus (Cat#: LVP430) into one well in 24-well-plate where cell density is at 50% ~ 75%. Image taken at ~72 hours after virus added (no medium changed). **Result:** The positive transduced cells are >90%.

#### Note: Filter wavelength settings:

GFP filter: ~Ex450-490 ~Em525;  
RFP filter: ~Ex545 ~Em620;  
CFP filter: ~Ex436 ~Em480;  
YFP filter: ~Ex500 ~Em535;

### Related Products:

<b>Product Category</b>	<b>Product Description</b> (please click links below to see product pages)
nuclear permeable CRE	<a href="#">Premade lentivirus for expressing nuclear permeable CRE recombinase with different fluorescent and different antibiotic selection markers.</a>
Luciferase expression	<a href="#">Premade lentivirus for Firefly-luciferase II, Renilla-luciferase, Gaussia-luciferase and Cypridina-luciferase with all different fluorescent and antibiotic markers.</a>



<b>iPS factors</b>	<a href="#">Premade lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FLF4) factors with different fluorescent and antibiotic markers</a>
<b>Human and mouse ORFs</b>	<a href="#">Premade lentivirus for <b>hundred of human and mouse ORFs</b> with RFP-Blastididin fusion dual markers.</a>
<b>Living cell imaging</b>	<a href="#">Pre-made lentivirus particles for Cell Organelle imaging for <b>Nucleus, Cytoplasm, Endoplasmic Reticulum, Golgi, Mitochondria, Nuclear membrane, Peroxisome, Plasma membrane, Microtubule, Chromatin, Annexin, Actin, Connexin, and more.</b></a>
<b>shRNA lentivirus</b>	<a href="#">Premade shRNA lentivirus for knockdown a specific genes (<b>P53, LacZ, Luciferase</b> and more).</a>
<b>Negative controls</b>	<a href="#">Premade <b>negative control lentivirus with different markers</b>: serves as the <b>negative control of lentivirals treatment</b>, for validation of the specificity of any <b>lentivirus target expression effects.</b></a>

### **Safety Precaution:**

GenTarget lentiviral particles adapt 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.