



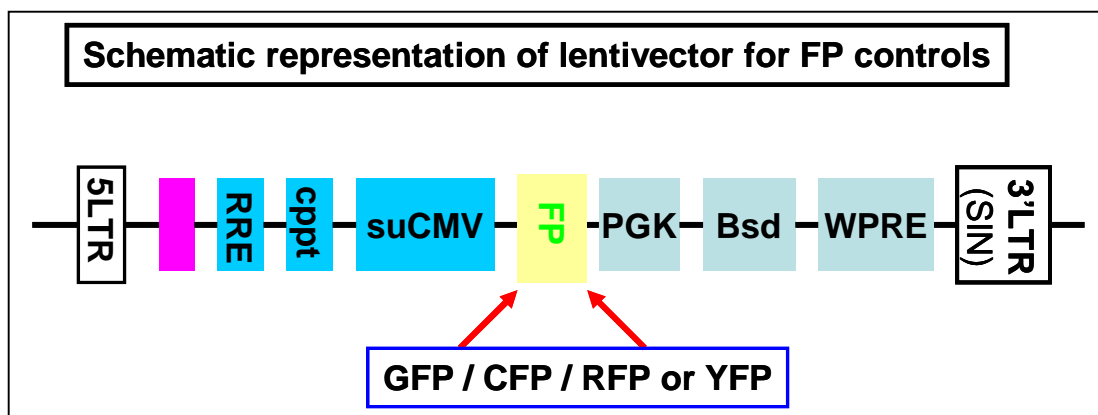
## Pre-made Lentiviral Particles for Fluorescent Proteins

Catalog#	Product Name	Amounts
LVP001	GFP Control Lentiviral particles	200ul x (1x10 <sup>8</sup> IFU/ml)
LVP001-SF	Serum free GFP Lentiviral particles	200ul x (1x10 <sup>8</sup> IFU/ml)
LVP001-PBS	GFP particles, in vivo ready	200ul x (1x10 <sup>9</sup> IFU/ml)
LVP011	CFP Control Lentiviral particles	200ul x (1x10 <sup>8</sup> IFU/ml)
LVP011-SF	Serum free CFP Lentiviral particles	200ul x (1x10 <sup>8</sup> IFU/ml)
LVP012	YFP Control Lentiviral particles	200ul x (1x10 <sup>8</sup> IFU/ml)
LVP012-SF	Serum free YFP Lentiviral particles	200ul x (1x10 <sup>8</sup> IFU/ml)
LVP023	RFP Control Lentiviral particles	200ul x (1x10 <sup>8</sup> IFU/ml)
LVP023-SF	Serum free RFP Lentiviral particles	200ul x (1x10 <sup>8</sup> IFU/ml)
LVP023-PBS	RFP particles, in vivo ready	200ul x (1x10 <sup>9</sup> IFU/ml)

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

### Product Description:

Lentiviral system is a gene delivery tool using lentivector for gene expression or knockdown. Lentivector is HIV-1 (Human Immunodeficiency Virus 1) derived plasmids. It produces lentiviral particles (lentivirus) that are capable to transduce into broad range of mammalian cell types or organs, including primary cells and non-dividing cells both in vivo and in cell culture system, and stably integrated into the transduced cell's genome, independent of cell cycle, for long term expression. Thus lentivirus holds unique promise as gene transfer agents.



Pre-made lentiviral particles for fluorescent proteins are generated from the proprietary lentiviral vector system. Each particles express codon optimized different fluorescent proteins which can be easily visualized under fluorescent microscope. Each fluorescent gene was first cloned into lentivector (see image above for lentivector's scheme). Then, 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 virus, packaged in either DMEM medium with 10% serum or serum-free medium without any human or animal components.

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

1. Regular particles in DMEM medium with 10% FBS and 60ug/ml polybrene;
2. Serum-free particles in serum-free medium without any human or animal origin components; they are best suitable for suspension cell transduction or for cultures that are sensitive to serum.
3. Concentrated and buffer exchanged particles in PBS as *in vivo ready* status.

Please See [FAQ for pre-made lentiviral particles](#) (.pdf) for more details.

## **Transduction Protocols:**

### **1. Adhesive cells Transduction Protocols:**

**Day 0:** Seed the cells in complete medium at appropriate density, incubate overnight.

(Note: at the time of transduction, it grows up to 10% ~50% confluent.)

**For example,** seed Hela cells at  $0.5 \times 10^5$ /ml x 0.5ml in a well of a 24-well plate;

**Day 1:** Remove the culture medium from the cells. Add fresh complete medium (Note: use as little media as possible at transduction). 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/CO2 incubator.

**For example,** add 50ul of lentiviral stock to the cells in 24-well plate above (getting MOI at 5).

**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 Quava machine).

**Day 3 + (optional):** Transduced cell can be sorted out via FACS, or selected by Blastcidin antibiotic. A pilot experiment should be done to determine the kill curve for your specific cell line, Bsd ranged from 0.5ug ~10ug/ml.

### **2. Suspension cells transduction Protocols:**

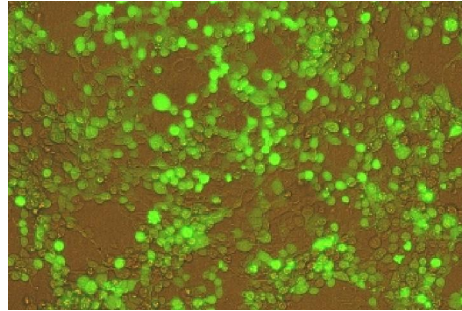
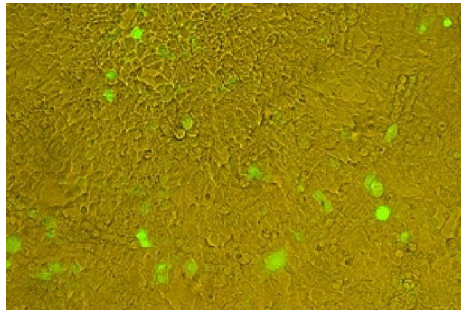
- ✿ Grow your cell in your completed suspension culture medium, shaking in flask in CO2 incubator;
- ✿ Measure cell density. When cell grow to  $\sim 3 \times 10^6$  cell/ml, measure cell viability (should > 90%), then diluted cells into  $1 \times 10^6$  cell/ml in completed medium;
- ✿ Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particle into the diluted cells at ratio of: **100ul virus per 1ml cells** (Note:



depend upon the cell types; you may need to use more or less viruses). Grow cells in flask, shaking in CO<sub>2</sub> incubator.

- ✿ At 24 hour after transduction, add equal amount of fresh medium containing final concentration of Blasticidin at 5 ~ 10ug/ml depend upon cell types. Grow cell shaking in CO<sub>2</sub> incubator. (Note: Gentarget's premade lentivirus contain Blasticidin resistance. So add Blasticidin antibiotic will enrich only the transduced cells for maximum protein production.)
- ✿ At 72 hours after transduction, check fluorescence under microscope or calculate the transduction efficiency using cell sorting machine (like FACS or Guava machine). (Note: GFP filter wavelength: Ex450-490 ~Em525; RFP filter: ~Ex545/~Em620).

### Transduction Example:



**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.

### Safety Precaution:

Please use extra caution when using lentiviral particles. Wear glove all the time at handling Lentiviral particles! Please refer CDC and NIH's links (see references) 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 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.