



## Pre-made Lentiviral Particles for SV40 large T-antigen

Cat#	Product Name	Amounts
<a href="#">LVP016-RB</a>	T-antigen ( <b>RFP-Bsd</b> ) lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP016-Neo</a>	T-antigen ( <b>Neo</b> ) lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP016-Bsd</a>	T-antigen ( <b>Bsd</b> ) lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP016-Puro</a>	T-antigen ( <b>Puro</b> ) lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP016-GB</a>	T-antigen ( <b>GFP-Bsd</b> ) lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP016-RP</a>	T-antigen ( <b>RFP-Puro</b> ) lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)

**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 almost 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 transduced cells' genome for long term expression. Therefore, lentivirus holds unique promise as gene transfer agents.

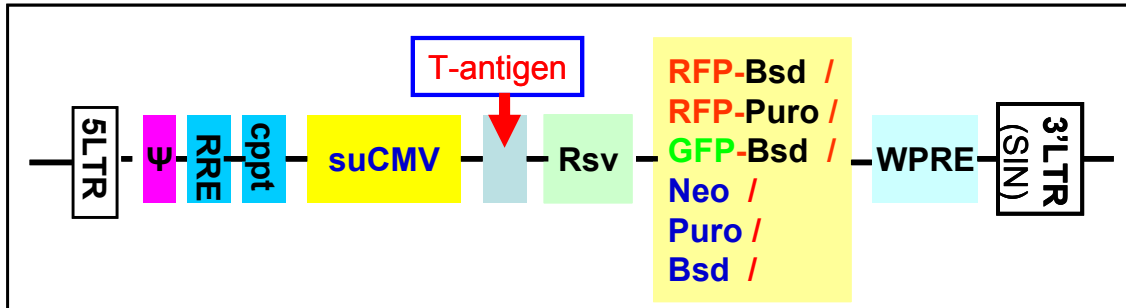
Pre-made large **T-antigen** lentiviral particles are generated from GenTarget's re-engineered lentivector system (see **vector map scheme** below). **T-antigen gene** was fully verified by sequencing. VSV-G pseudotyped lentiviral particles are generated in 293T cell, and packaged in DMEM medium with 10% serum or in serum free medium, supplied as 200ul/per vial at 1 x 10<sup>7</sup> IFU/ml. For general questions about our ready-to-use particles, please see **FAQ for pre-made lentiviral particles** (.pdf) on our website (<http://www.gentarget.com/pdf/FAQ-Premade-Lentiviral-particles.pdf>).

### **Key features:**

1. Each lentiviral particles contain a specific resistant marker, used for selecting the transduced cells or generating stable cell lines by antibiotics selection or via fluorescent cell sorting.
2. Target was expressed natively without any tag. (note: His-tagged T-antigen is also available upon special request.)



3. The strongest suCMV promoter make the pre-made virus a ideal tool for mammalian protein expression, stable cell line construction and enzymatic assays both in vivo or in vitro (see schematic vector map below).
4. The lentivirus are ready and easy to use, simply add 50ul into one well culture in 24-well plate.



## Transduction Protocols:

### 1. Adhesive cells Transduction Protocols:

**Day 0:** Seed the desired cells in complete medium at appropriate density incubate overnight. (Note: at the time of transduction, it grows to 25% ~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:** Thaw the Pre-made lentiviral stock at room temperature. Add appropriate amount of virus stock to obtain the desired MOI. Or simply add 50 ul of virus into one well in 24-well plate without worry about the MOI number. Return cells to 37°C/CO<sub>2</sub> incubator. **A common used MOI is 10.**

**Day 3:** At the time of ~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). (**Note:** some cell lines need longer time, up to one week to see the transduction effects / the fluorescent signal.)

**Day 3 + (optional):** Transduced cell can be sorted out via FACS. Or you can select transduced stable cell line by a specific antibiotic (dependent upon the used particles types). A pilot experiment should be done to determine the antibiotic kill curve for your specific cell line.

**Note: Filter wavelength settings:**

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



## 2. Suspension cells transduction Protocols:

1. Grow your cell in your completed suspension culture medium, shaking in flask in CO2 incubator;
2. 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;
3. Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particle into the diluted cells at ratio of: **100ul virus per ml cells** (Note: depend upon the cell types; you may need to use more or less viruses). Grow cells in flask, shaking in CO2 incubator.
4. 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 CO2 incubator. (Note: Gentarget's premade lentivirus contain Blasticidin resistance. So add Blasticidin antibiotic will enrich only the transduced cells for maximum protein production.)
5. At 72 hours after transduction, check fluorescence under microscope or calculate the transduction efficiency using cell sorting machine (like FACS or Guava machine).

### Safety Precaution:

Please use extra caution when using lentiviral particles. Remember. 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. [NIH stem cell training program \(Link\)](#).
2. Takahashi, K. and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663-676.
3. Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R., Slukvin, I.I., and Thomson, J.A. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318, 1917-1920.
4. Park, I.H., et al., Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*, 2008. 451(7175): p. 141-6.
5. Shao, L., et al., Generation of iPS cells using defined factors linked via the self-cleaving 2A sequences in a single open reading frame. *Cell Res.*, 2009. 19(3): p. 296-306.
6. NIH Guidelines for [Biosafety Considerations for Research with Lentiviral Vectors](#). (Link).
7. [CDC guidelines for Lab Biosafety levels \(Link\)](#).

### Warranty:

This product 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.