



## Pre-made Lentiviral Particles for Target Overexpression (for human, mouse or rat genes / ORFs)

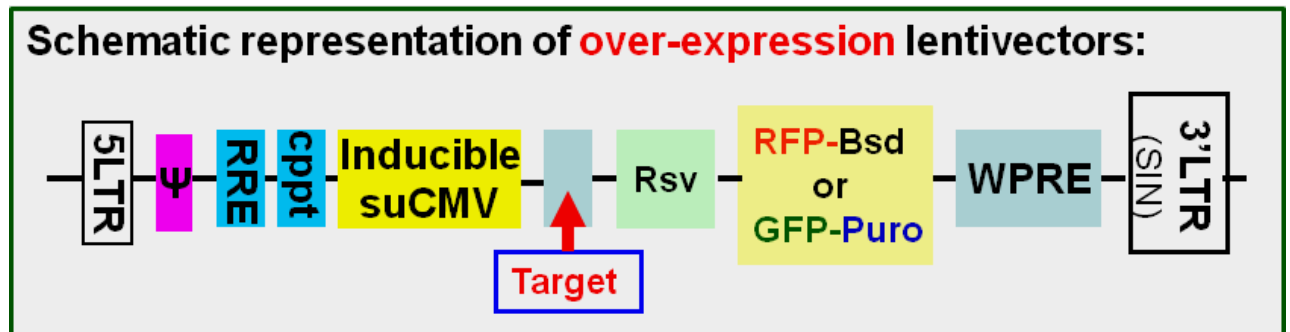
**Amount:** 200ul/vial ( $1 \times 10^7$  IFU/ml), or concentrated in PBS ( $1 \times 10^{8-9}$  IFU/ml).

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

### Product Description:

GenTarget's Lentiviral gene delivery system is Human Immunodeficiency Virus-1 (HIV) based lentivector plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into almost 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 for specific human or mouse genes are generated from GenTarget's **optional inducible lentiviral system** (see vector scheme below). The vector used to produce these viral particles includes a self-inactivation feature in its 3' LTR, causing it to only generate replication-incompetent particles.



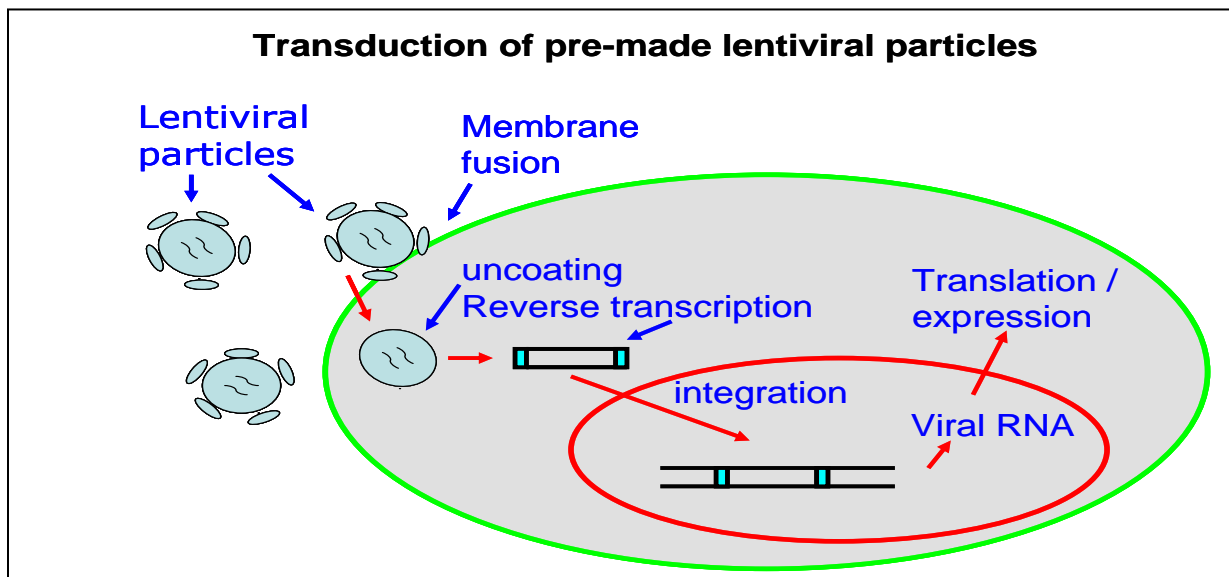
Each particle expresses a fully sequence-verified human, mouse, or rat target matching the CDS sequence in NCBI (see all product list in [Product List table](#) at end of this manual). The human targets were natively expressed under a **tetracycline-inducible suCMV promoter**. A **blasticidin-RFP or GFP-Puromycin** fusion dual marker under an RSV promoter allows sorting or selection of transduced cells by fluorescent signal or antibiotic resistance, as desired. The RFP or GFP signal provides a convenient, real-time means to monitor the particles' performance.



All inducible lentiviral particles can be used for regular constitutive expression, and can optionally be used for tetracycline-inducible expression in the presence of the tetracycline repressor protein (TetR). For inducible expression, the target expression is first repressed by TetR, and then induced with the addition of tetracycline. The presence of TetR can be achieved by co-infection with [premade TetR lentiviral particles](#) or co-transfection with a TetR expression plasmid, or simply by using a [TetR expressing stable cell line](#). Please see our website for more information about the [inducible lentiviral system](#). GenTarget provides [TetR lentiviral particles](#) with a variety of antibiotic selection markers for double selection of the target expressing cells.

GenTarget also provides [Negative Control Lentivirus](#) for establishing mock lentivirus treatment in a given cell line. The negative control lentivirus also provides a means to validate the specificity of any target expression effects. The control virus, CAT# [CMV-Null-RB](#), has a lentivector backbone identical to that of the target expression virus, but expresses only the dual marker.

These ready-to-use particles are packaged in 293T cells and provided as a 200 µl aliquot (Note: the particles can be provided in PBS on special request). Particles are safe and easy to use; simply add them into cultured cells or organs. Each lot of particles is validated and target expression is guaranteed. Please see our [FAQs about premade lentiviral particles](#).





## Key features:

- High target expression levels driven by extremely strong suCMV promoter
- Easy transduction monitoring that can be readily verified by the RFP signal
- **Optional** tetracycline-inducible expression if desired
- Dual markers: transduced cells can be sorted on the RFP signal or selected by blasticidin resistance
- The lentivirus is ready to use; simply add it into your cell culture **(see transduction carton image above)**.
- **Ready to use:** simply add lentivirus into cell culture. No need any other reagents.

## Transduction Protocols:

### **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  $\mu$ l 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  $0.5 \times 10^5$ /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<sub>2</sub> 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  $-80^{\circ}\text{C}$  for future use; virus titer will decrease by  $\sim 10\%$  for each freeze/thaw cycle.

### Day 3:

At  $\sim 72\text{hr}$  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:**

Grow cells in complete suspension culture medium; use a shaking flask in a  $\text{CO}_2$  incubator if necessary.

Measure cell density. When density has reached  $\sim 3 \times 10^6$  cells/ml, measured viability should be  $> 90\%$ . Dilute cells into  $1 \times 10^6$  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  $\mu\text{l}$  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  $\text{CO}_2$  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  $\text{CO}_2$  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.



## 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.

## Attachment: GenTarget's Pre-made lentivirus Products:

| <b>Product Category</b>                             | <b>Product Description<br/>(please click category name to see product's pages)</b>  |
|---|---|
| <a href="#">Fluorescent markers</a>                 | Preamde lentivirus express human codon optimized fluorescent protein, <b>GFP / RFP / CFP / BFP / YFP</b> .                                  |
| <a href="#">Luciferase expression</a>               | Premade lentivirus for all kinds of luciferase protein expression: <b>firefly and Renilla</b> with different antibiotic selection markers.  |
| <a href="#">CRE recombinase</a>                     | Premade lentivirus for expressing <b>nuclear permeant CRE</b> recombinase with different flurescent and antibiotic markers.                 |
| <a href="#">LoxP ColorSwitch</a>                    | Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.                       |
| <a href="#">CRISPR /hu CAS9</a>                     | Preamde lentivirus express humanzied wild-type <b>Cas9</b> endonuclease for genomic editing with <b>CRISPR</b>                              |
| <a href="#">TetR inducible expression repressor</a> | Premade lentivirus expressin <b>TetR</b> (tetracycline regulator) protein, the repressor protein for the inducible expression system.       |
| <a href="#">iPS factors</a>                         | Premde lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FLF4</b> ) factors with different fluorescent and antibitoic markers |



|   |  |
|---|--|
| <a href="#">T-antigen Expression</a>                  | Express <b>SV40 large T antigen</b> with different selection markers   |
| <a href="#">Cell Organelle imaging</a>                | Premade lentivirus for cell organelle imaging. The fluorescent marker <b>GFP/RFP/CFP was sub-cellular localized</b> in different cell organelle for living cell imaging.                                     |
| <a href="#">LacZ expression</a>                       | Express different full length <b><math>\beta</math>- galactosidase (lacZ)</b> with different selection markers   |
| <a href="#">Anti-miRNA lentivirus</a>                 | Pre-made lentivirus expression a specific <b>anti-miRNA</b> cassette.  |
| <a href="#">Fluorescent-ORF fusion</a>                | Pre-made lentivirus expression a " <b>GFP/RFP/CFP-ORF</b> " fusion target.   |
| <a href="#">Pre-made shRNA lentivirus</a>             | Premade shRNA lentivirus for knockdown a specific genes ( <b>P53, LacZ, Luciferase</b> and more).  |
| <a href="#">microRNA and anti-microRNA lentivirus</a> | Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human and mouse miRNA.   |
| <a href="#">Negative control lentiviruses</a>         | Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentiviruses treatment, for validation of the specificity of any lentivirus target expression effects. |
| <a href="#">Other Enzyme expression</a>               | Ready-to-use lentivirus, expressing <b>specific enzymes</b> with different selection markers.  |