



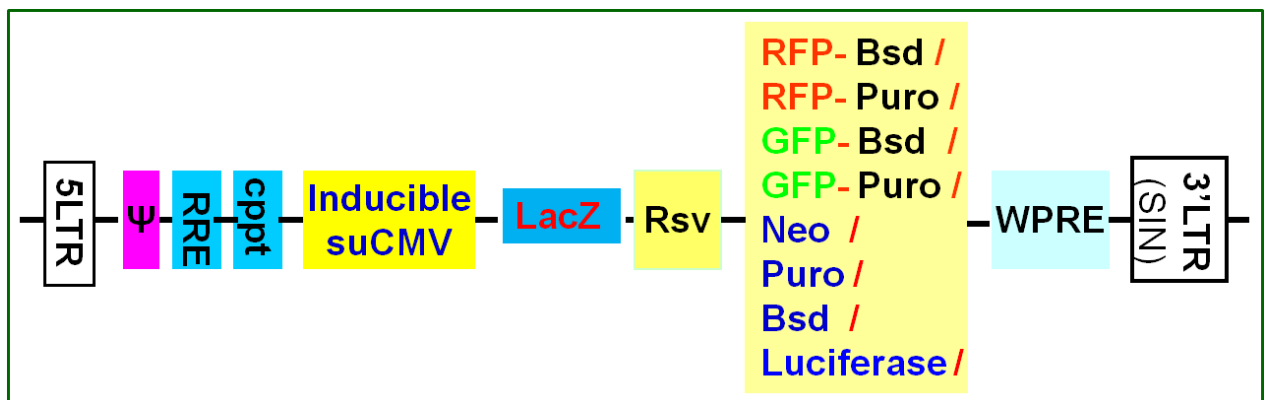
## Pre-made lentivirus for $\beta$ -Galactosidase expression

Cat#	Product Name	Amounts
<a href="#">LVP010</a>	lacZ ( <b>RFP-Bsd</b> ) lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
<a href="#">LVP301</a>	lacZ ( <b>Puro</b> ) lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
<a href="#">LVP333</a>	lacZ ( <b>Neo</b> ) lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
<a href="#">LVP334</a>	lacZ ( <b>luciferase</b> ) lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
<a href="#">LVP346</a>	lacZ ( <b>Bsd</b> ) lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
<a href="#">LVP347</a>	lacZ ( <b>GFP-Bsd</b> ) lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
<a href="#">LVP348</a>	lacZ ( <b>RFP-puro</b> ) lentiviral particles	1x10 <sup>7</sup> IFU/ml x 200ul
<a href="#">LVP021</a>	<a href="#">GFP-lacZ (His) Lentiviral particles</a>	1x10 <sup>7</sup> IFU/ml x 200ul
<a href="#">LVP021-PBS</a>	<b>GFP-lacZ</b> (His) Concentrated Lentivirus in PBS	5 x10 <sup>7</sup> IFU/ml x 200ul

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

### Product Description:

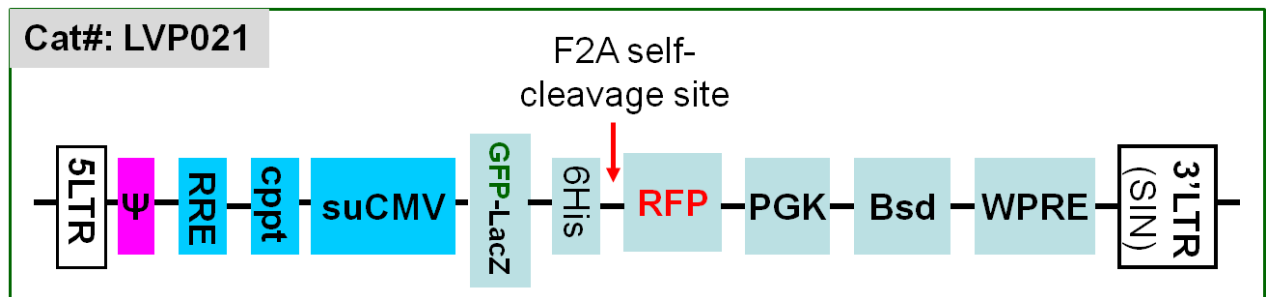
GenTarget’s lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into almost all kinds of mammalian cells, including stem cells, primary cells, and non-dividing cells both *in vivo* and *in vitro*. Lentiviral Particles stably integrate into the transduced cells’ genome for long term expression, making it a great gene transfer agent.





Pre-made **LacZ** lentiviral particles are generated from GenTarget's **Optional inducible lentiviral system** with different selection markers (see vector core structure scheme above). Full length **LacZ ORF** was fully verified by sequencing. VSV-G pseudotyped lentiviral particles are generated in 293T cell, and provided in **DMEM medium** with 10% FBS, 60ug/ml of polybrene, For more details about premade particles, please see **FAQ for pre-made lentiviral particles** (.pdf). (<http://www.gentarget.com/pdf/FAQ-Premade-Lentiviral-particles.pdf>)

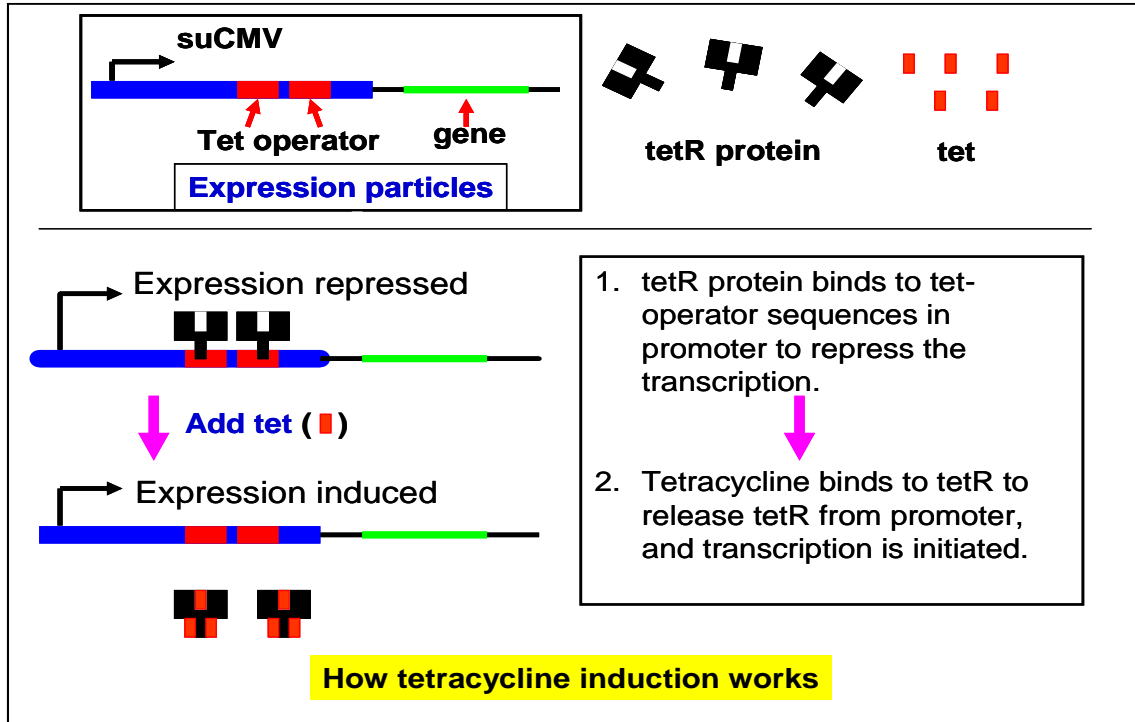
For product Cat#: LVP021, the full-length Beta galactosidase (lacZ) was fused with N-term GFP protein and with C-term a 6His under an inducible CMV promoter. A RFP protein was bicistronically expressed under the same CMV promoter, mediated via a F2A element. (see vector map scheme below). So it has triple signals: GFP, RFP signals that can be visualized via microscope, and lacZ signal via staining. Please see the scheme for core lentivector structure:



### **About inducible expression:**

LacZ was natively expressed (without any tags) under a tetracycline inducible suCMV promoter in which two tetracycline operator sequences was integrated. However, the particles can be used for regular constitutive high expression without requirements for tetracycline induction. It becomes inducible expression particles only when the tetracycline **repressor protein (tetR)** is present in advance. For inducible expression, the tetR must be expressed in advance to stop the transcription, and the expressed was activated by adding tetracycline. This inducible expression is tetracycline's dose dependent. In general, the amount of tetracycline is used at 1.0-10 ug/ml final concentration. The image below illustrates how the inducible expression works.

Gentarget provides "**premade tetR particles**" with different antibiotics for double selecting the transduced inducible expression cells.



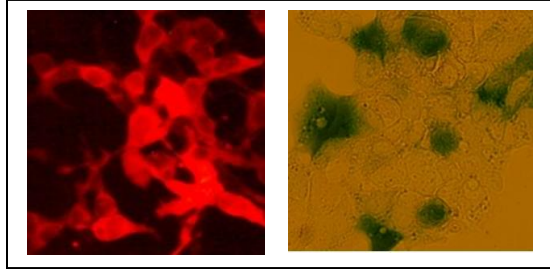
- Particles are used in a tetR expression stable cell line that constantly express tetR protein in advance;
- Transfect a tetR expression plasmid before transduce lentiviral particles;
- Co-transduce both the tetR repressor particles and the gene expression particles into the sample cells (with equal MOI) and the double transduced cells can be selected by both antibiotics, and then used for inducible expression.

### Key features:

1. High LacZ expression level and high viral titer;
2. Easy transduction monitoring via the RFP or GFP fluorescent signal under microscope (not available for all particles);
3. Dual markers: transduced cells can be sorted via a fluorescent signal or selected via antibiotics (not available for all particles);
4. **The lentivirus are ready and easy to use, simply add 50ul into your cell culture in 24-well plate (see sample image below).** (Note: dependent upon your specific needs, you may design the transduction with different MOI for different levels of expression.)



## Transduction sample image:



**Figure 1:** 50 ul of pre-made lentiviral particles (Cat#: LVP010) added into 293HEK cells. At 72 hours after transduction, image was taken under microscope with RFP filter (Left image), then cells washed with PBS and stained with lacZ staining kit for 10min, take the bright light image to see lacZ stained cells (Right image) (**note:** prolonged staining time should show all lacZ positive cells which is not showed here).

## 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 °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 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 CO<sub>2</sub> incubator if necessary.

Measure cell density. When density has reached ~3 x 10<sup>6</sup> cells/ml, measured viability should be > 90%. Dilute cells into 1 x 10<sup>6</sup> 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 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<sub>2</sub> 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<sub>2</sub> 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.

**Note: Filter wavelength settings:**

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

**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. Molecular Therapy (2003) 7, 460-466; doi: 10.1016/S1525-0016(03)00024-8
2. Annu Rev Microbiol. 1994;48:345-69.
3. Microbiol Mol Biol Rev. 2005 Jun;69(2):326-56.
4. NIH Guidelines for [Biosafety Considerations for Research with Lentiviral Vectors](#). (Link).
5. [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.

**Related Products:** GenTarget's pre-made lentivirus product category.

<b>Lentivirus Category</b> (click to see)	<b>Product Description</b>
<a href="#">Target Expression</a>	Premade lentivirus express a <b>human, mouse or rat</b> gene with Fluorescent-Antibiotic fusion dual selection.
<a href="#">Luciferase expression</a>	Premade lentivirus express all kinds of luciferase: <b>firefly; Renilla; Cypridina; Red-Luc; Nano-Luc</b> , with different fluorescent and antibiotic selection.
<a href="#">Fluorescent markers</a>	Preamde lentivirus express human codon optimized fluorescent protein, <b>GFP / RFP / CFP / BFP / YFP / niRFP / unstable GFP, etc.</b>
<a href="#">Cytoskeleton Imaging</a>	Fluorescent ( <b>GFP / RFP / CFP</b> ) labelled cell skeleton protein (Actin; Tubulin; Paxillin; Vimentin)
<a href="#">Cell Organelle imaging</a>	Premade lentivirus for cell organelle imaging. The fluorescent labelled cell organelle lentivirus for living cell



	imaging.
<a href="#">CRISPR /hu CAS9</a>	Preamde lentivirus express humanized wild-type <b>Cas9</b> endonuclease for genomic editing by <b>CRISPR</b>
<a href="#">Fluorescent Fusion target</a>	Lentivirus express the " <b>Fluorescent-Target</b> " fusion proteins. A desired target is fused to <b>Green, Blue, Red,</b> or <b>Cyan</b> Fluorescent Protein, demonstrating the target's functionality and localization
<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- <b>GFP</b> -Stop-LoxP- <b>RFP</b> " cassette, used to monitor the CRE recombination event in vivo.
<a href="#">SEAP Reporter</a>	<b>SEAP</b> (Secreted Embryonic Alkaline Phosphatase) secreted expression lentivirus under different promoter.
<a href="#">TetR repressor expression</a>	Premade lentivirus expressin <b>TetR</b> (tetracycline regulator) protein, the repressor protein for the inducible expression system.
<a href="#">rtTA Expression</a>	Lentivirus express the reverse tetraccycline transcription activator gene, rtTA-M2 with different selection.
<a href="#">Pathway Reporter</a>	Different Report lentivirus ( <b>Luc, RFP, GFP, SEAP</b> ) under a pathway specific response promoter.
<a href="#">Cell Immortalization</a>	Comprehesive lentivirus for cell immortalization, for different cell types.
<a href="#">Cell Specific reporter</a>	Different Report lentivirus driven by cell specific promoter.
<a href="#">Infectious Antigens</a>	Lentivirus express all kinds of infectious antigens.
<a href="#">Viral Like Particle (VLP)</a>	Lentiviral particles pseudo-typed with high density of surface envelope protein.
<a href="#">Immuno Therapy</a>	Lentivirus products for Immuno Therapy application.
<a href="#">iPS factors</a>	Premde lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FLF4</b> ) factors with different fluorescent and antibiotic markers
<a href="#">LacZ expression</a>	Express different full length <b><math>\beta</math>- galactosidase (lacZ)</b> with different selection markers
<a href="#">Anti-miNA lentivirus</a>	Pre-made lentivirus expression a specific <b>anti-miRNA</b> cassette.



<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</a>	Ready-to-use lentivirus, expressing <b>specific enzymes</b> with different selection markers.