



Pre-made Lentiviral Particles for β -Galactosidase manual

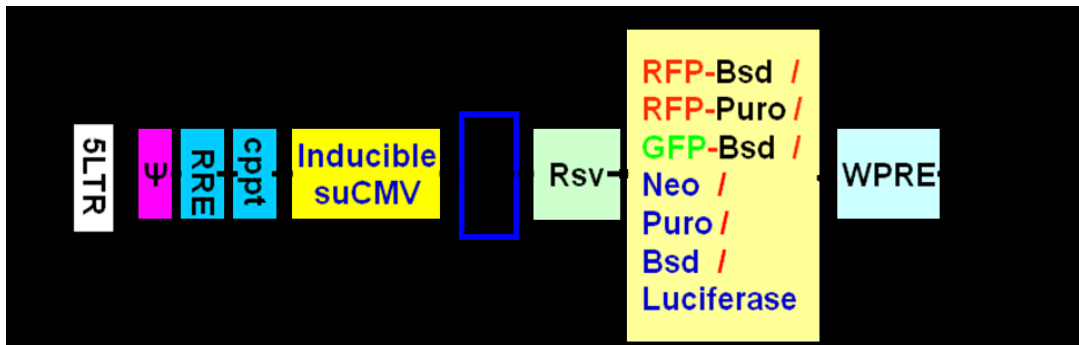
Cat#	Product Name	Amounts
LVP010	lacZ (RFP-Bsd) lentiviral particles	1×10^7 IFU/ml x 200ul
LVP301	lacZ (Puro) lentiviral particles	1×10^7 IFU/ml x 200ul
LVP333	lacZ (Neo) lentiviral particles	1×10^7 IFU/ml x 200ul
LVP334	lacZ (luciferase) lentiviral particles	1×10^7 IFU/ml x 200ul
LVP346	lacZ (Bsd) lentiviral particles	1×10^7 IFU/ml x 200ul
LVP347	lacZ (GFP-Bsd) lentiviral particles	1×10^7 IFU/ml x 200ul
LVP348	lacZ (RFP-puro) lentiviral particles	1×10^7 IFU/ml x 200ul
LVP021	GFP-lacZ (His) Lentiviral particles	1×10^7 IFU/ml x 200ul

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

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 **LacZ** lentiviral particles are generated from GenTarget's [Optional inducible lentiviral system](#). (see vector scheme below).

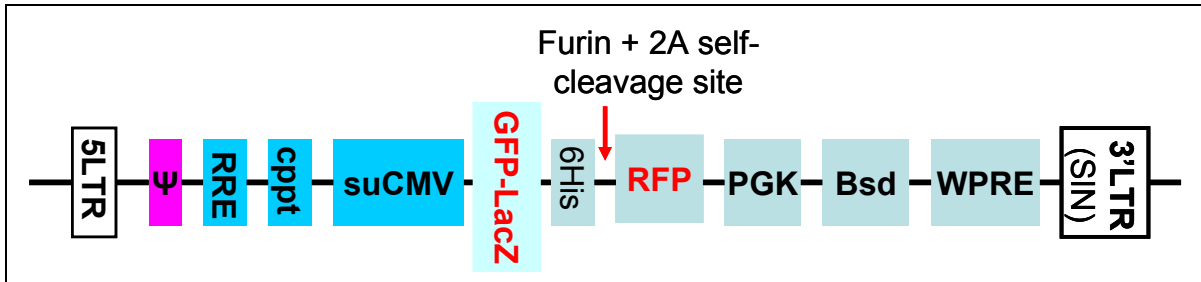


Full length [LacZ ORF](#) was fully verified by sequencing. VSV-G pseudotyped lentiviral particles are generated in 293T cell, and provided in **DMEM medium** with 10% FBS, 60ug/ml of polybrene, For more details about premade particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf). (<http://www.gentarget.com/pdf/FAQ-Premade-Lentiviral-particles.pdf>)



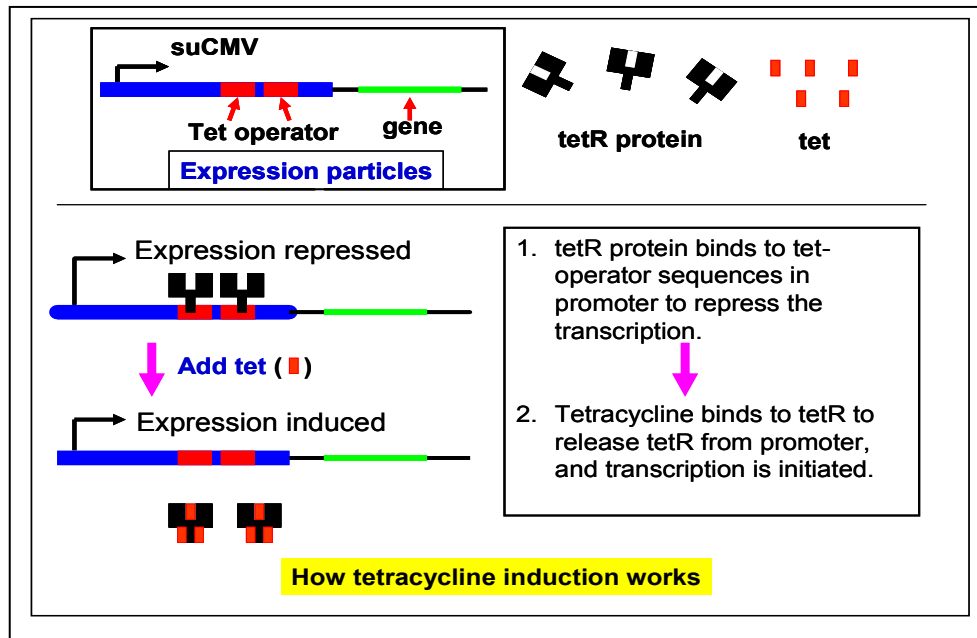
For product Cat#: LVP021, the full-length Beta galactosidase (lacZ) was fused with N-term GFP protein and with C-term a 6His under an inducible CMV promoter. A RFP protein was bicistronically expressed under the same CMV promoter, mediated via a F2A element. (see vector map scheme below). So it has triple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

Vector map scheme (for Cat#: LVP021 only):



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 regulator 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 1ug/ml final concentration. The image below illustrates how the inducible expression works.





- If inducible expression is desired, the repressor regulator (tetR) expression must be delivered in advance or at the same time for transduction. Gentarget provides “[premade tetR particles](#)” with different antibiotics for double selecting the transduced cells.

The presence of tetR can be achieved by the following methods:

- 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:

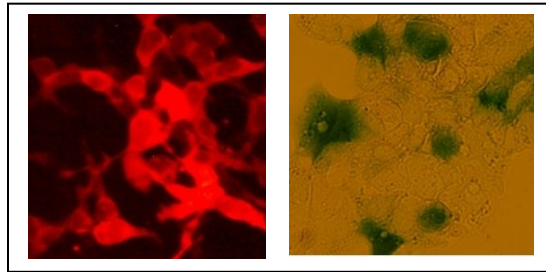


Figure 1: 50 ul of pre-made lentiviral particles (Cat#: LVP010) added into 293HEK cells. At 72 hours after transduction, image was taken under microscope with RFP filter (Left image), then cells washed with PBS and stained with lacZ staining kit for 10min, take the bright light image to see lacZ stained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

Transduction Protocols:

1. 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 $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$ 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/CO₂ incubator. **A common used MOI is 10.**

Day 3: At ~72hr post 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 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 1: **A quick application 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 cell by antibiotics.

Note 2: For some cell types such as primary cells – It may take up to longer time for maximal expression; in some cases, maximal expression may not be detected until 1 week post-transduction.

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 1×10^6 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: **200ul virus per 2ml cells** (Note: depend upon the cell types; you may need to use more 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 the antibiotic dependent upon the particles. Grow cell shaking in CO2 incubator.
5. At at 72 hours, 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. 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:

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