



Pre-made Lentiviral Particles for Flp recombinase manual

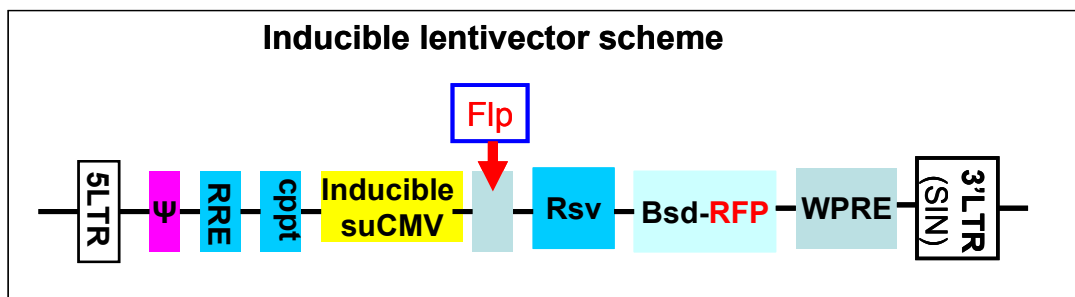
Cat#	Product Name	Amounts
LVP014	Flp lentiviral particles	200ul, ~1 x 10 ⁷ IFU/mL in DMEM containing 10% FBS and 60ug/ml polybrene

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 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 **Flp** lentiviral particles are generated from GenTarget's [inducible lentiviral system](#). (see vector scheme below). [Flp recombinase gene](#) was fully verified by sequencing. VSV-G pseudotyped lentiviral particles are generated in 293T cell, and provided in in DMEM containing 10% FBS and 60ug/ml of polybrene. For more details about premade particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf).

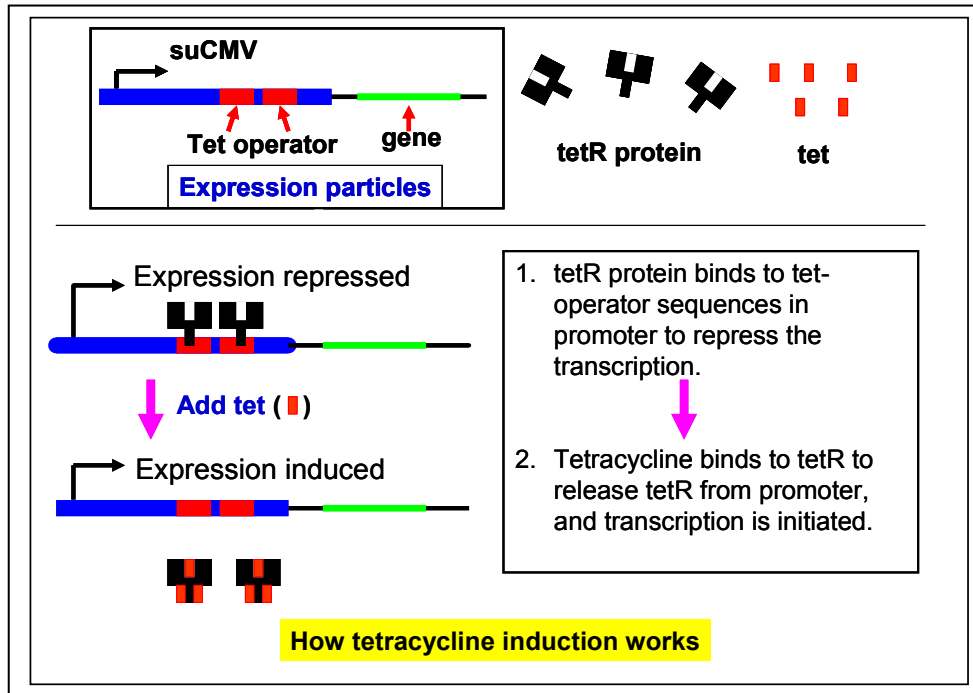


Inducible expression:

Flp recombinase 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. 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. Gentarget provides “**premade tetR particles**” with different antibiotics for double selecting the transduced cells.

Key features:

1. High Flp expression level and high viral titer;
2. Easy transduction monitoring via the RFP fluorescent signal under microscope;
3. Dual markers: transduced cells can be sorted via a RFP fluorescent signal or selected via blasticidin antibiotic;
4. **The lentivirus are ready and easy to use, simply add 50ul into your cell culture in 24-well plate.** (Note: dependent upon your specific needs, you may design the transduction with different MOI for different levels of expression.)



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 grow to 10% ~50% confluent.)

For example, seed Hela cells at 0.5×10^5 /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 5ul ~ 50ul of lentiviral stock to the cells in 24-well plate above (getting MOI from 0.5 to 5).

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).

Day 3 + (optional): Transduced cell can be sorted out via FACS. Or you can select transduced stable cell line by Blastcidin. 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:

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 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 Blastcidin at 5 ~ 10ug/ml depend upon cell types. Grow cell shaking in CO2 incubator. (Note: Gentarget's premade lentivirus contain Blastcidin resistance. So add Blastcidin 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). (Note: GFP filter wavelength: Ex450-490 ~Em525; RFP filter: ~Ex545/~Em620).



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Safety Precaution:

Please use extra caution when using lentiviral particles. Remember. Wear gloves 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. OGorman et al., 1991; Sauer, 1994).
2. Molecular Therapy (2003) 7, 460-466;
3. Annu Rev Microbiol. 1994;48:345-69.
4. Microbiol Mol Biol Rev. 2005 Jun;69(2):326-56.
5. NIH Guidelines for [Biosafety Considerations for Research with Lentiviral Vectors](#). (Link).
6. [CDC guidelines for Lab Biosafety levels \(Link\)](#).

Warranty:

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