



Pre-made Lentiviral Particles for Flp recombinase manual

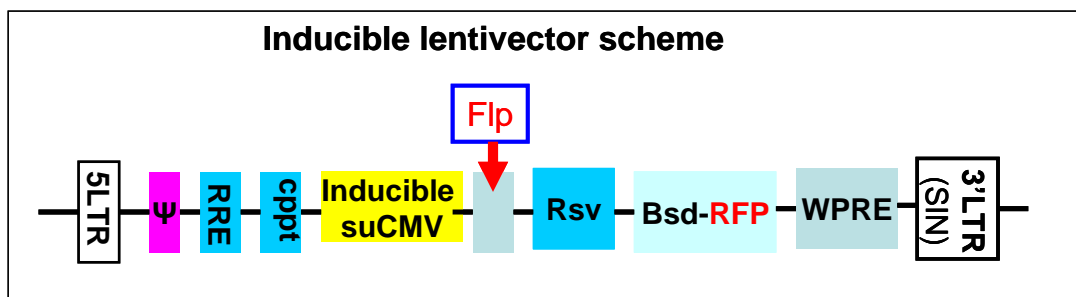
Cat#	Product Name	Amounts
LVP014	Flp lentiviral particles	200ul, $\sim 1 \times 10^7$ IFU/mL in DMEM containing 10% FBS and 60ug/ml polybrene

Storage: $< -70^\circ\text{C}$, avoid repeat freeze/thaw cycles. Stable for 6 months at $< -70^\circ\text{C}$.

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 **Flp** lentiviral particles are generated from GenTarget's [Optional 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 DMEM containing 10% FBS and 60ug/ml of polybrene. For more details about premade particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf).

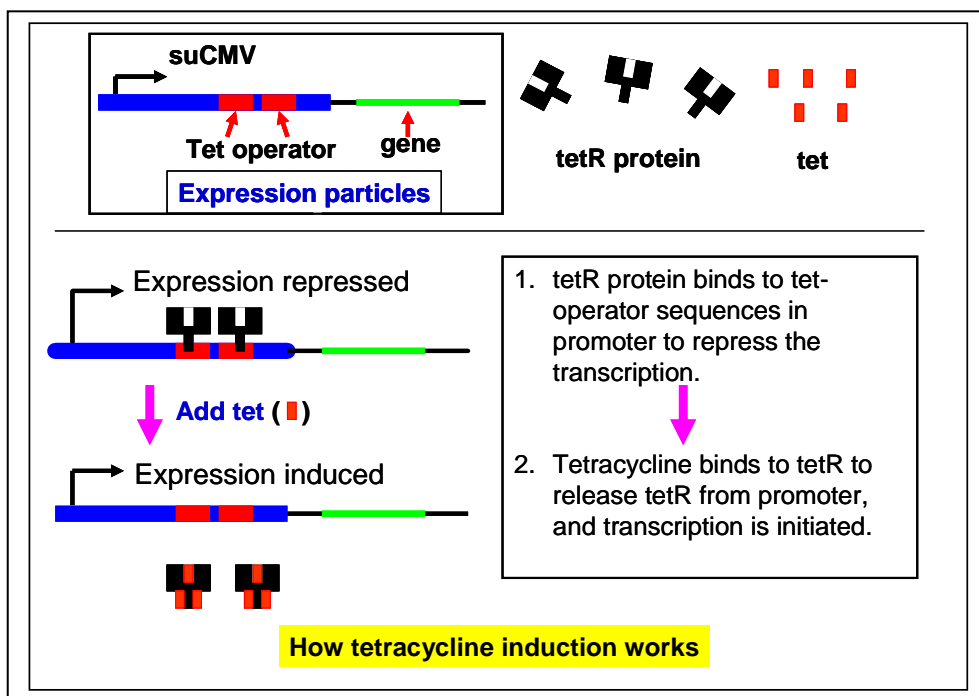


Inducible expression:

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. 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 μ 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 $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$ in a well of a 24-well plate.

Day 1:

- Thaw the pre-made lentiviral stock at room temperature and add the appropriate amount of virus stock to obtain the desired MOI.
- Return cells to 37°C, CO₂ incubator. Do nothing.

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 48hr~72hr (Depend upon cell type) 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, or select by antibiotic killing. 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 CO₂ incubator if required.

Measure cell density (not grow over 3 million/ml), measured viability should be > 90%. Dilute cells into 1 x 10⁶ 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 CO₂ incubator.

Day 2:

At 24 hours after transduction, add an equal amount of fresh medium containing. Continue growing cells in CO₂ incubator.

Day 3+:

At 48 hour to 72 hours (Depend upon cell type) after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Pass cells into 0.5 million/ml density in completed medium containing the corresponding antibiotic (**Note:** amount of antibiotic depends on cell type. A killing curve must pre-established). Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

Safety Precaution:

Gentarget lentiviral particles adapts 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 at handling Lentiviral particles!



Please refer CDC and NIH's guidelines 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:

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.

Attachment: GenTarget's pre-made lentivirus product categories.

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Lentivirus for all kinds of pathway assays
Cell Immortalization	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.
ImmunoOncology Research	Lentivirus products for immuno therapy research, CAR-T, TCR-T, Assay cell lines, and Cell Antigens & Receptors.
CRISPR Gene Editing	Preamde lentivirus express humanized wild-type Cas9 endonuclease, the dCas9 , gRNAs, CRISPR gene editing research
Cell-Specific Reporter	a set of reporter lentiviruses to express a luminescence or fluorescent reporter (firefly Luciferase, Renilla luciferase, RFP or GFP fluorescent marker) under a tissue specific promoter
Infectious Antigens	Lentivirus that express all kinds of infectious antigens with C-term 6His-tag.
Virus Like Particles (VLP)	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
Non-integrating LV	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.



shRNA Knockdown	Knockdown verified and customized shRNA lentivirus for target knockdown,
microRNA lentivirus	Premade lentivirus expression human or mouse precursor miRNA . And anti-miRNA lentivector and virus for human and mouse miRNA.
Anti-miRNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Human and mouse ORFs	Premade lentivirus expressing a human, mouse or rat gene with RFP-Blasticidin fusion dual markers.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression: firefly and Renilla, Red-Luc and more , with different antibiotic selection markers.
Fluorescent Markers	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, mRFP, unstable GFP and others.
Luminescent Imaging	Lentivirus express Nano-Lantern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
Cytoskeleton Imaging	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
Unstable GFP	Lentivirus express the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection
near-infrared RFP	The near-infrared Red fluorescent (niRFP) expression Lentivirus provides the whole-body images with better contrast and brighter images
Fluorescent-ORF fusion	Pre-made lentivirus expression a " GFP/RFP/CFP-ORF " fusion target.
CRE recombinase	Premade lentivirus for expressing nuclear permeant CRE recombinase with different fluorescent and antibiotic markers.
LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
SEAP Reporter	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
TetR Repressor	Premade lentivirus expressing TetR (tetracycline



	regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF inducible system.
iPS factors	Premade lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FLF4) factors with different fluorescent and antibiotic markers
LacZ expression	Express different full length β-galactosidase (lacZ) with different selection markers
Negative control lentiviruses	Premade negative control lentivirus with different markers : serves as the negative control of lentivirus treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.
Ultra titer lentivirus	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.