



Pre-made Lentiviral Particles for nuclear permeant CRE recombinase expression

Cat#	Product Name	Amounts
LVP336	NLS-CRE (Bsd) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP336-PBS	NLS-CRE (Bsd) lentiviral particles , in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP339	NLS-CRE (Puro) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP339-PBS	NLS-CRE (Puro) lentiviral particles , in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP297	NLS-CRE (Neo) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP297-PBS	NLS-CRE (Neo) lentiviral particles , in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP013	NLS-CRE-2A-RFP (Bsd) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP013-PBS	NLS-CRE-2A-RFP (Bsd) lentiviral particles , in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP338	NLS-CRE-2A-RFP (Puro) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP338-PBS	NLS-CRE-2A-RFP (Puro) lentiviral particles , in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP027	NLS-CRE-2A-RFP (Neo) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP027-PBS	NLS-CRE-2A-RFP (Neo) lentiviral particles , in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP337	NLS-CRE-2A-GFP (Bsd) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP337-PBS	NLS-CRE-2A-GFP (Bsd) lentiviral particles , in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP407	NLS-CRE-2A-GFP (Puro) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP407-PBS	NLS-CRE-2A-GFP (Puro) lentiviral particles , in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP408	NLS-CRE-2A-GFP (Neo) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP408-PBS	NLS-CRE-2A-GFP (Neo) lentiviral particles, in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP304	Luciferase-2A-NLS-CRE (Bsd) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS



LVP304-PBS	Luciferase-2A-NLS-CRE (Bsd) lentiviral particles, in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP409	Luciferase-2A-NLS-CRE (Puro) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP409-PBS	Luciferase-2A-NLS-CRE (Puro) lentiviral particles, in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP410	Luciferase-2A-NLS-CRE (Neo) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP410-PBS	Luciferase-2A-NLS-CRE (Neo) lentiviral particles, in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP411	Luciferase-2A-NLS-CRE (GFP-Bsd) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP411-PBS	Luciferase-2A-NLS-CRE (GFP-Bsd) lentiviral particles, in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP412	Luciferase-2A-NLS-CRE (GFP-Puro) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP412-PBS	Luciferase-2A-NLS-CRE (GFP-Puro) lentiviral particles, in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP413	Luciferase-2A-NLS-CRE (RFP-Bsd) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP413-PBS	Luciferase-2A-NLS-CRE (RFP-Bsd) lentiviral particles, in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS
LVP414	Luciferase-2A-NLS-CRE (RFP-Puro) lentiviral particles	1x10 ⁷ IFU/ml x 200ul in DMEM with 10% FBS
LVP414-PBS	Luciferase-2A-NLS-CRE (RFP-Puro) lentiviral particles, in vivo ready	5 x10 ⁷ IFU/ml x 200ul In PBS

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

1. 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,

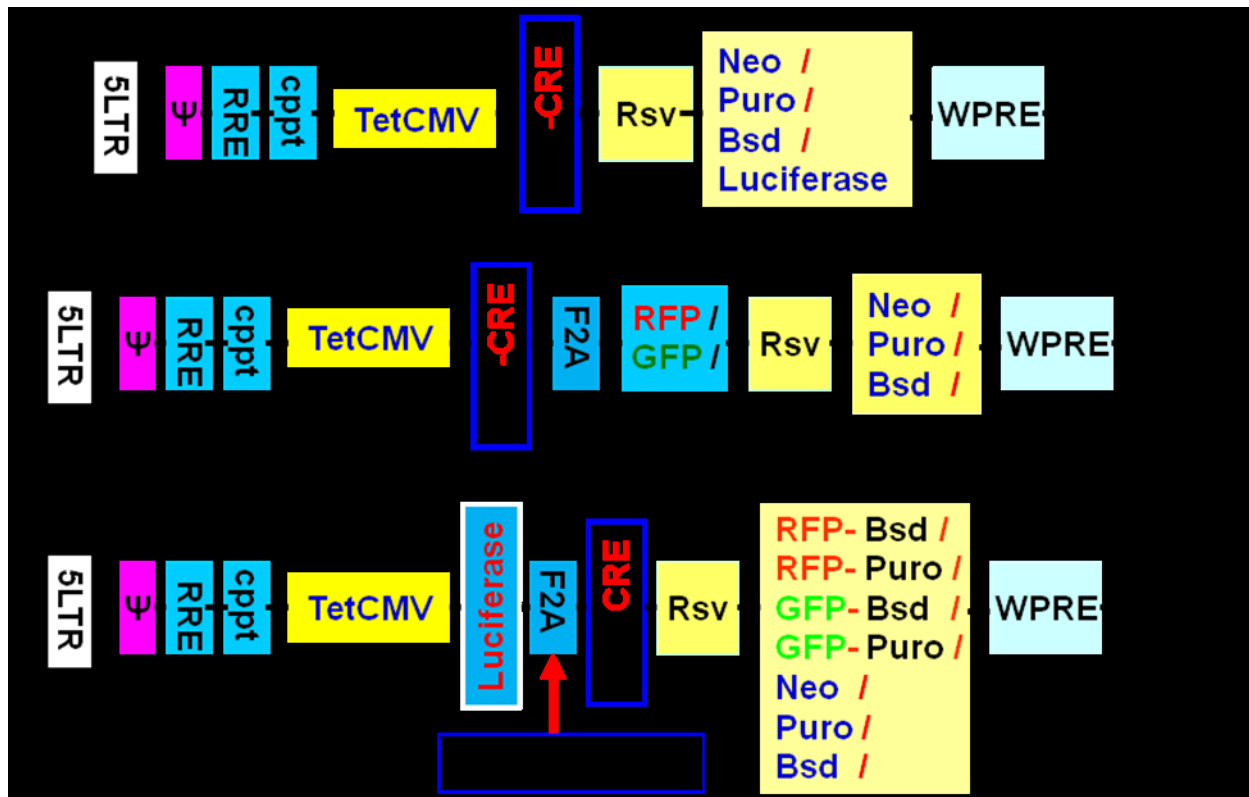


primary cells and non-dividing cells both *in vivo* and in **cell culture** system. Particles stably integrate into transduced cells' genome for long term expression. Therefore, lentivirus holds unique promise as gene transfer agents.

CRE protein, from bacteriophage P1, catalyzes recombination between 34 base pair target sequences, named lox sites. CRE lentiviral particles can catalyze the joining event *In Vivo* in the presence of lox sites. Purified CRE enzyme can joint individual plasmids containing lox sites.

Gentarget provides premade, CRE expression lentiviral particles a **nuclear localization signal (NLS)**, PKKKRKV, from the [SV40 Large T-antigen](#), at its N-terminal, which allows CRE penetrate nuclear membrane. This nuclear permeant CRE increase the recombination events *in vivo*.

To satisfy all kinds of needs, GenTarget CRE expression lentivirus was provided with **different fluorescent markers, antibiotic markers, or fluorescent-antibiotic fusion dual markers**. We also provides CRE, luciferase and fluorescent protein (GFP/RFP) **triple labeled** lentivirus. Please see vector schemes below for the each expression vector structure.





Pre-made **CRE recombinase** lentiviral particles are generated from GenTarget's **Optional inducible lentiviral system, or SureTiter™ Lentiviral System**. **CRE gene** (click to see its sequence) was fully verified by sequencing analysis. In the cases of CRE and a marker was bicistronically expressed under the same CMV promoter, CRE and the marker were expressed as individual protein (not as fusion), which was mediated by a self-cleavage element (F2A element) through a translation skipping mechanism. VSV-G pseudotyped particles are generated from 293T cells, filtered through 0.45mm filter and titer validated in lot-by-lot basis.

Ready-to-use CRE lentiviral particles are provided in two formats:

- packaged in **DMEM containing 10% FBS** and 60ug/ml of polybrene (10X);
- particles were concentrated and buffer exchanged in **PBS**, do not contain any additives. It is best suitable for serum sensitive culture or *in vivo* applications and for hard-to-transduced cell lines.

For more details about premade particles, please see **FAQ for pre-made lentiviral particles** (.pdf).

2. About the inducible expression:

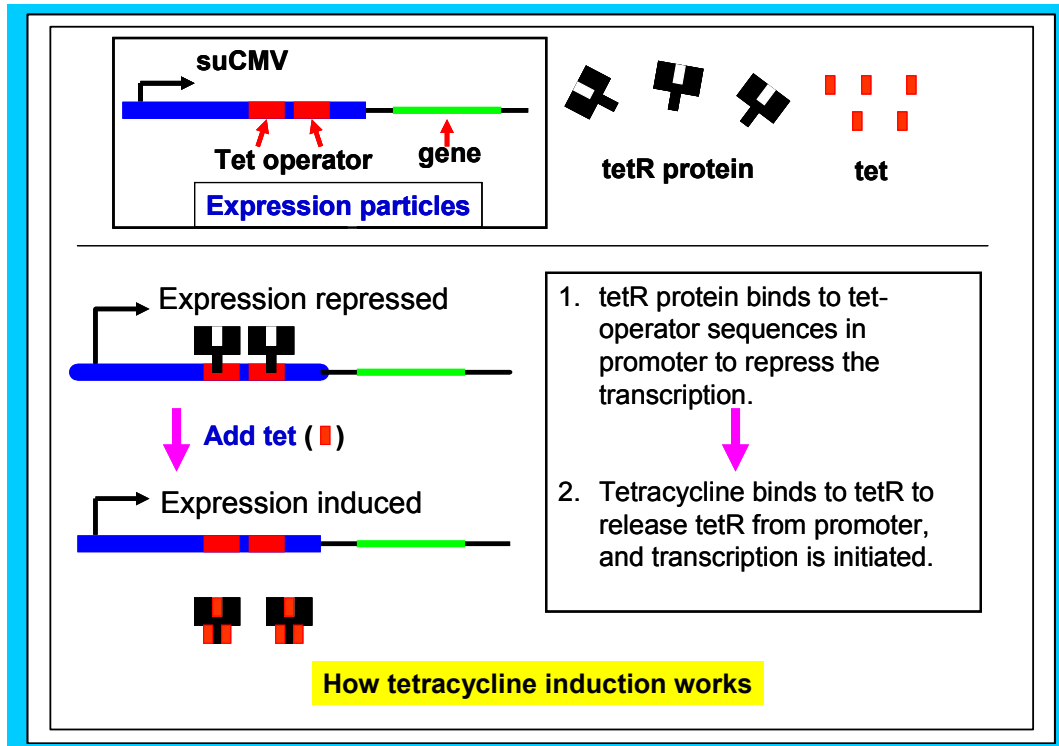
All particles can be used for constitutive high expression of CRE without need any induction. However, CRE (or with a bicistronic maker) was expressed under an optional tetracycline inducible suCMV promoter. So those particles can also be **optionally** used as tetracycline induced expression when the tetracycline regulator protein (tetR) is present in advance. The tetR must be expressed in advance to stop the particles' transcription, and the expressed was activated by adding tetracycline (see the picture below for details). 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 desirable, 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:

- tetR is already expressed in a 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.



3. Key features:

1. High nuclear permeant CRE expression level and high viral titer;
2. Easy transduction monitoring via the GFP or RFP fluorescent signal under microscope (not for all products);
3. Dual markers: transduced cells can be sorted via a fluorescent signal or selected via antibiotics (not for all products);
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.)

4. Transduction Protocols (for reference only):

1. Adhesive cells Transduction Protocols:

Note: 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), pass cell into antibiotic containing medium, or sort the cells via fluorescent signal.



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: 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.

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 its specific antibiotics (dependent upon the antibiotic types). A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line. (Refer to any literatures about How to generate stable cell lines.)

2. Suspension cells transduction Protocols:

- Grow your cell in your completed suspension culture medium, shaking in flask in CO₂ incubator;
- 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;
- 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.
- At 24 hour after transduction, add equal amount of fresh medium containing related antibiotics (Note: each particles contain an antibiotic marker and the antibiotic amounts to use is depend upon cell types). Grow cell shaking in CO₂ incubator.
- At 72 hours after transduction, check fluorescence under microscope or calculate the transduction efficiency using cell sorting machine (like FACS or Guava machine).
- You can sort the fluorescent positive cells or keep selection the antibiotic resistant cell to generate a stable cell lines.

Note: Filter wavelength settings:

GFP filter: ~Ex450-490 ~Em525;
RFP filter: ~Ex545 ~Em620;
CFP filter: ~Ex436 ~Em480;
YFP filter: ~Ex500 ~Em535;

5. 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. 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 [Bio-safety Considerations for Research with Lentiviral Vectors](#). (Link).
5. [CDC guidelines for Lab Bio-safety 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:

Product Category	Product Description (please click links below to see product pages)
CRE reporter cell line	"Color stwich" Stable cell lines expresses "loxP-GFP-Stop-Loxp-RFP" cassette, used to monitor CRE recombination efficiency.
Fluorescent controls	Premade control lentivirus for Fluorescent protein control lentivirus: GFP, RFP, YFP and CFP with different antibiotic selection markers
Luciferase expression	Premade lentivirus for firefly-luciferase II, Renilla-luciferase, Gaussia-luciferase and Cyprinda-luciferase with all different flurescent and antibiotic markers.
iPS factors	Premde lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FLF4) factors with different fluorescent and antibitoic markers
Human and mouse ORFs	Premade lentivirus for hundred of human and mouse ORFs with RFP-Blastididin fusion dual markers.
Living cell imaging	Pre-made lentivirus particles for Cell Organelle imaging for Nucleus, Cytoplasm, Endoplasmic Reticulum, Golgi, Mitochondria, Nuclear membrane, Peroxisome, Plasma membrane, Microtubule, Chromatin, Annexin, Actin, Connexin, and more.
shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (P53, LacZ, Luciferase and more).
Negative controls	Premade negative control lentivirus with different markers: serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression effects.



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