



Pre-made Lentivirus for Nuclear Permeant CRE Recombinase Expression

Cat#	Product Name	Amounts
<u>LVP336-PBS</u>	CRE (CMV Promoter, Bsd), Concentrated Lentivirus	200ul x (1 x10 ⁸ IFU/ml) in PBS solution, premixed with 10x Polybrene (60ug/ml)
<u>LVP339-PBS</u>	CRE (CMV Promoter, Puro), Concentrated Lentivirus	
<u>LVP297-PBS</u>	CRE (CMV Promoter, Neo), Concentrated Lentivirus	
<u>LVP013-PBS</u>	CRE-2A-RFP (CMV, Bsd), Concentrated Lentivirus	
<u>LVP338-PBS</u>	CRE-2A-RFP (CMV, Puro), Concentrated Lentivirus	
<u>LVP027-PBS</u>	CRE-2A-RFP (CMV, Neo), Concentrated Lentivirus	
<u>LVP337-PBS</u>	CRE-2A-GFP (CMV, Bsd) Concentrated Lentivirus	
<u>LVP407-PBS</u>	CRE-2A-GFP (CMV, Puro), Concentrated Lentivirus	
<u>LVP408-PBS</u>	CRE-2A-GFP (CMV, Neo), Concentrated Lentivirus	
<u>LVP804-PBS</u>	CRE-2A-GFP (CMV promoter), Concentrated Lentivirus	
<u>LVP805-PBS</u>	CRE-2A- RFP (CMV promoter), Concentrated Lentivirus	
<u>LVP519-PBS</u>	CRE (EF1a Promoter, Bsd), Concentrated Lentivirus	
<u>LVP520-PBS</u>	CRE (EF1a Promoter, Puro), Concentrated Lentivirus	
<u>LVP521-PBS</u>	CRE (EF1a Promoter, Neo), Concentrated Lentivirus	
<u>LVP522-PBS</u>	CRE-2A-RFP (EF1a Pro, Bsd), Concentrated Lentivirus	
<u>LVP523-PBS</u>	CRE-2A-RFP (EF1a Pro, Puro), Concentrated Lentivirus	



<u>LVP524-PBS</u>	CRE-2A-RFP (EF1a Pro, Neo) , Concentrated Lentivirus
<u>LVP525-PBS</u>	CRE-2A-GFP (EF1a Pro, Bsd) , Concentrated Lentivirus
<u>LVP526-PBS</u>	CRE-2A-GFP (EF1a Pro, Puro) , Concentrated Lentivirus
<u>LVP527-PBS</u>	CRE-2A-GFP (EF1a Pro, Neo) , Concentrated Lentivirus
<u>LVP573-PBS</u>	CRE (CAG promoter, Puro) , Concentrated Lentivirus
<u>LVP574-PBS</u>	CRE CAG promoter, Bsd , Concentrated Lentivirus
<u>LVP575-PBS</u>	CRE (CAG promoter, Neo) , Concentrated Lentivirus
<u>LVP576-PBS</u>	CRE (GFP-Puro) (CAG Promoter), Concentrated lentivirus
<u>LVP577-PBS</u>	CRE (RFP-Bsd) , (CAG Promoter), Concentrated lentivirus
<u>LVP578-PBS</u>	CRE (RFP-Puro) , (CAG Promoter), Concentrated lentivirus
<u>LVP304-PBS</u>	Luciferase-2A-CRE (CMV Pro, Bsd), Concentrated Lentivirus
<u>LVP409-PBS</u>	Luciferase-2A-CRE (CMV Pro, Puro), Concentrated Lentivirus
<u>LVP410-PBS</u>	Luciferase-2A-CRE (CMV Pro, Neo), Concentrated Lentivirus
<u>LVP411-PBS</u>	CMV-Luciferase-2A-CRE (GFP-Bsd), Concentrated Lentivirus
<u>LVP412-PBS</u>	CMV-Luciferase-2A-CRE (GFP-Puro), Concentrated Lentivirus
<u>LVP413-PBS</u>	CMV-Luciferase-2A-CRE (RFP-Bsd), Concentrated Lentivirus
<u>LVP414-PBS</u>	CMV-Luciferase-2A-CRE (RFP-Puro), Concentrated Lentivirus

Storage: -80 °C, avoid repeat freeze/thaw cycles. Stable for 12 months.



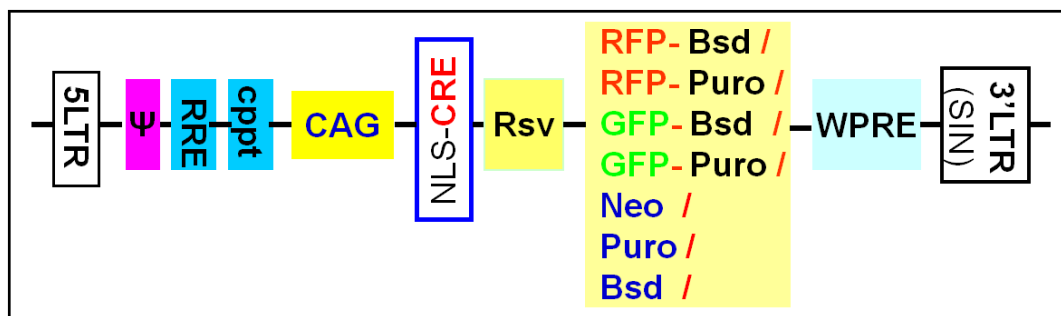
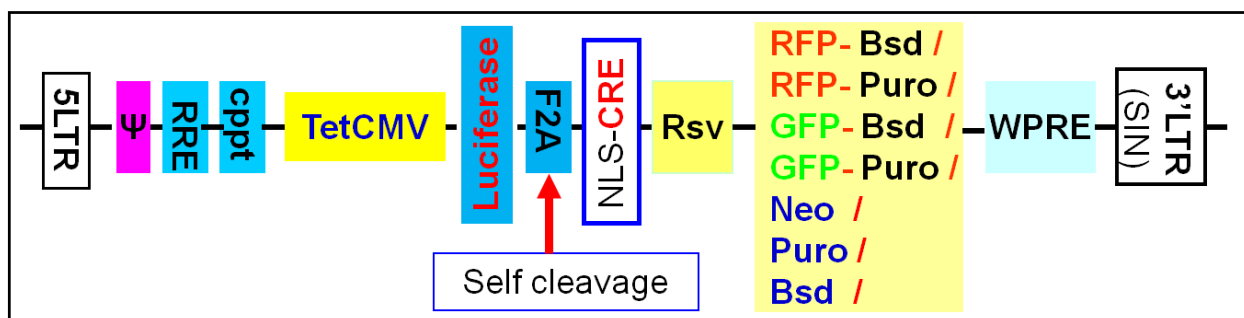
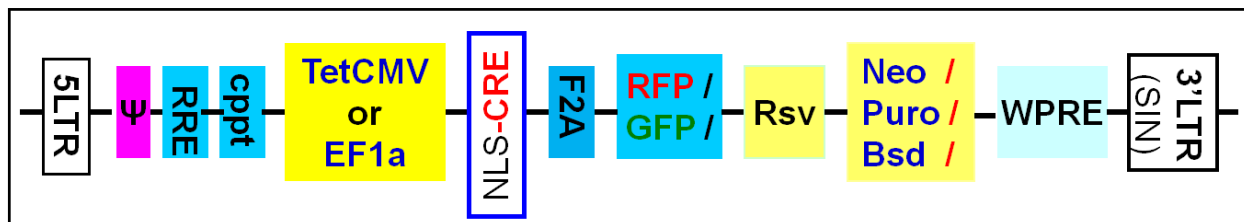
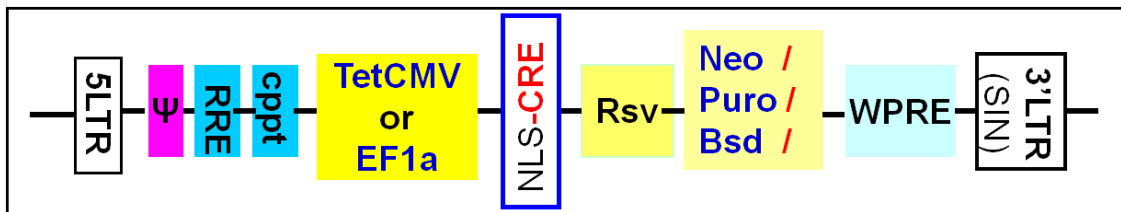
1. Product Description:

GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate Lentivirus(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*. Lentivirus stably integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.

CRE recombinase, from bacteriophage P1, catalyzes recombination between 34 base-pair target sequences called lox sites and can join individual plasmids containing lox sites. CRE recombination provides an excellent tool for conditional gene targeting studies in transgenic animal models by linking genotypic alterations to biological outcomes (phenotypes). GenTarget provides premade, expression-ready CRE Lentivirus for *in vivo* and *in vitro* use. CRE expressed by these particles contains the **nuclear localization signal (NLS)**, PKKKRKV from the [SV40 Large T-antigen](#) at its N-terminus, allowing penetration of the nuclear membrane and thereby increasing the number of *in vivo* recombination events.

GenTarget's [CRE recombinase](#) is expressed under either an [optional inducible CMV promoter](#) (TetCMV), an enhanced constitutive **EF1a** promoter, or a **CAG** promoter with **a variety of fluorescent markers, antibiotic markers, or fluorescent-antibiotic fusion dual markers**. We also provide CRE-expressing lentivirus "**triple-labeled**" with luciferase, an antibiotic resistance marker, and a fluorescent protein. Some of the lentiviruses express CRE and a marker bicistronically under the same promoter as individual proteins (rather than fusion proteins) through a translation skipping mechanism that mediated by a self-cleavage (F2A) element. Some product (CAT#:[LVP804](#)) does not contain any antibiotic marker.

Please see the vector schemes below for each expression vector structure. Pre-made CRE lentiviruses are generated from GenTarget's [Optional Inducible Lentiviral System](#), or [SureTiter™ Lentiviral System](#).



VSV-G pseudotyped particles are generated from 293T cells and passed through a 0.45 mm filter. Titer is validated for each lot.

For more details, please see [FAQs for pre-made lentiviral particles \(.pdf\)](#).

2. About the promoters:

EF1a is strong promoter in all cell types, and has a low likelihood of being silenced in long term cell culture. It is **not** an inducible promoter; it constitutively expresses CRE in all cell types.

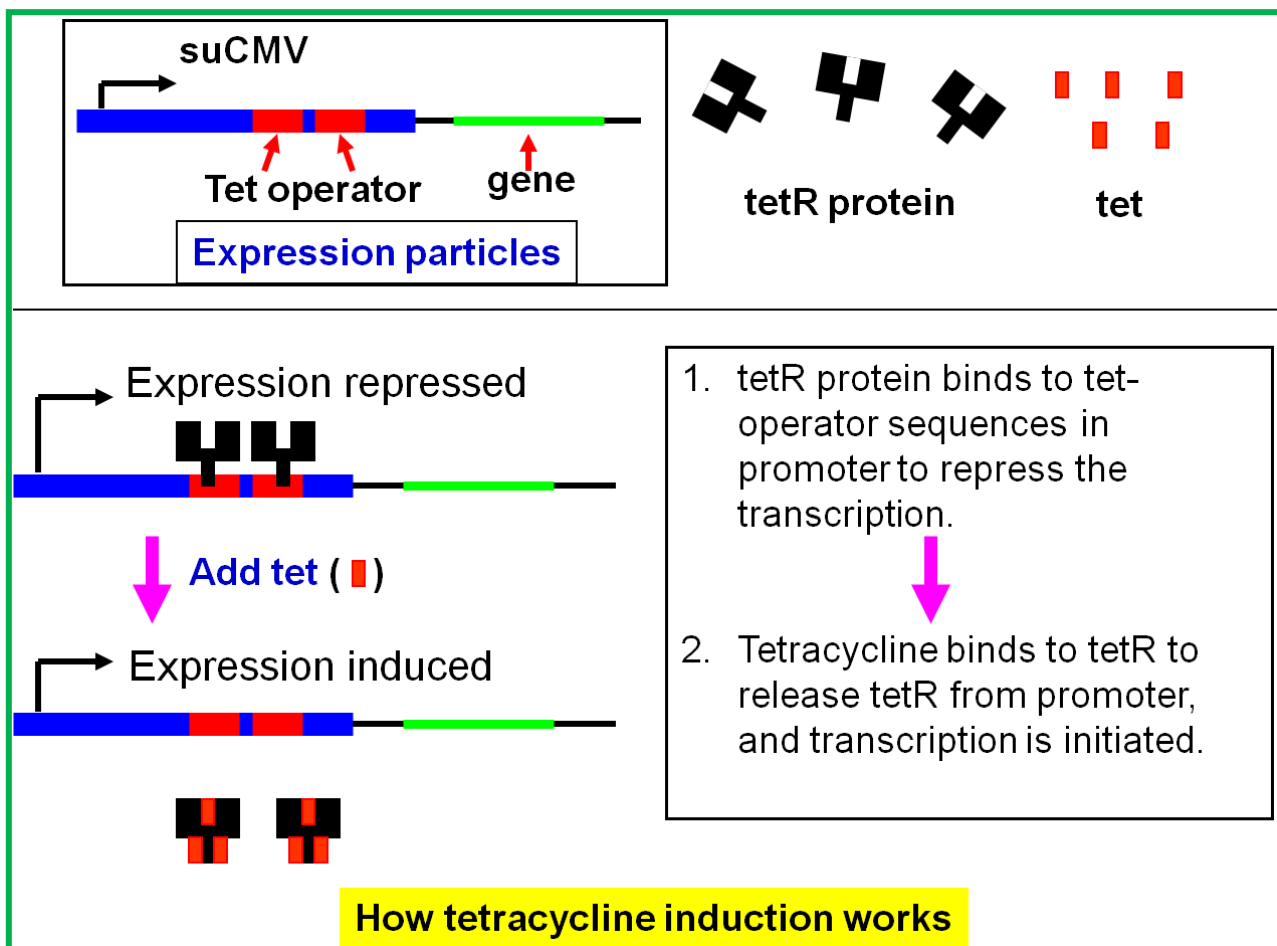


The optional inducible CMV promoter (**TetCMV**) can also constitutively express high levels of CRE without any induction. It is the strongest promoter in most cell lines; however, it may be silenced after long-term culture in some lines. The TetCMV promoter is embedded with two copies of the repressor binding sequence (TetO). Consequently, the TetCMV can be used for **optional** tetracycline-induced expression. For inducible expression, the TetR repressor protein must be expressed in advance to stop CMV-driven expression. Expression can then be activated by the addition of tetracycline (see the picture below for details). Inducible expression is tetracycline dose-dependent; in general, a final tetracycline concentration of 1.0~5.0 µg/ml is used. The image below illustrates how inducible expression works.

The **CAG** promoter is a combination of the cytomegalovirus (CMV) early enhancer element and the chicken beta-actin promoter. It is the strongest promoter in embryonic stem (ES) cells and is frequently used to drive high level gene expression in mouse ES cells.

If inducible expression is desired, repressor regulator (TetR) expression must be delivered in advance of or at the same time as transduction. The presence of TetR can be achieved by the following methods:

- 1) **TetR stable cell lines** that constitutively express the TetR protein
- 2) **Co-transfection** with a TetR expression plasmid and a target-inducible expression vector
- 3) **Co-transduction** with TetR Lentivirus and inducible gene expression lentiviral particles



GenTarget provides “[premade TetR particles](#)” with different antibiotics for double selecting the transduced cells.

3. Key features of CRE expression lentivirus:

- 1) **High expression levels** of nuclear permeant CRE and high viral titer
- 2) **A selection of promoters** to meet your needs
- 3) **Easy transduction monitoring** of the GFP or RFP signal by fluorescence microscopy (not all products)
- 4) **Dual markers:** transduced cells can be sorted via a fluorescent signal or selected for antibiotic resistance (not for all products)
- 5) **Ready to use:** simply add 50 µl into your cell culture in a 24-well plate. (**Note:** depending upon your specific needs, you may transduce with different MOIs for different levels of expression.)



4. Transduction Protocols:

Note: Pre-made lentivirus is provided ready to use status, simply added into your cell culture. The amount of lentivirus to add depends on cell type. In general, add 50 μ l of virus into one well in 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 if applicable. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection. (**Note:** for suspension cells or the "hard-to-transduced" cell type, you may need to double the virus amount added.)

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).

Note: Filter wavelength settings:

GFP filter: ~Ex450-490 ~Em525;

RFP filter: ~Ex558 ~Em583;



5. Safety Precaution:

GenTarget Lentivirus 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 Lentivirus 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.

6. References:

- 1) Molecular Therapy (2003) 7, 460–466; doi: 10.1016/S1525-0016(03)00024-8
- 2) Nucleic Acids Research, 2001, V29, No12 e56;
- 3) Annu Rev Microbiol. 1994;48:345-69.
- 4) Microbiol Mol Biol Rev. 2005 Jun;69(2):326-56.
- 5) NIH Guidelines for [Bio-safety Considerations for Research with Lentiviral Vectors](#). (Link).
- 6) [CDC guidelines for Lab Bio-safety levels \(Link\)](#).

7. Warranty:

This product is for research use only. It is warranted to meet its quality as described when used in 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.

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

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Repoter Lentivirus for all kinds of pathway screening 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 and TCR; Assay Cell Lines for T-cell targeted killing assay and other cell-based assays; over-expression lentivirus products for the immune response targets; Cell surface antigens (CDs); immune checkpoint / Receptors; CRISPR gene Repair and knock-IN lentivirus; CRISPR knockout lentivirus;
CAR-T, TCR Lentivirus	CARs Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; TCRs : MART-1/ NY-ESO1/



Product Category	Product Description (please click into each category's page)
	CD1d- α -GalCer/ TR α V3-F2A-TR β V5-6;
CRISPR Gene Editing	Preamde lentivirus express humanized wild-type Cas9 endonuclease, the dCas9 , gRNAs, CRISPR gene editing research
Epigenomic: CRISPRi and CRISPRa	" dCas9-Protein " fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
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



Product Category	Product Description (please click into each category's page)
	dynamic processes in living cells and organisms
Sub-cellular Imaging	Lentivirus contain a well-defined organelle targeting signal fused to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways.
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 Lentiviruses 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.
CRE, Flp ColorSwitch	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or "FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the CRE or Flp 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, FLK4) factors with different fluorescent and antibiotic markers
LacZ expression	Express different full length β-galactosidase (lacZ) with different selection markers
	Premade negative control lentivirus with different



GenTarget Inc

7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Fax: 1 (800) 380-4198
Email: orders@gentarget.com

Product Category	Product Description (please click into each category's page)
Negative control lentiviruses	markers: serves as the negative control of lentiviruses 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.