GenTarget Inc

7930 Arjons Drive, Suite B
San Diego, CA 92126
Phone: (858) 6788683
Fax: (800) 3804198
Email: orders@gentarget.com

Pre-made tetracycline regulator (TetR) expression lentiviral particles

CAT#	Product Name	Amounts
LVP017-GB	CMV-TetR (GFP-Bsd) Lentiviral particles	200ul, (1 x 10^7 IFU/mL) in DMEM medium containing 10% FBS and 10x polybrene (60ug/ml)
LVP017-RB	CMV-TetR (RFP- Bsd) Lentiviral particles	
LVP017-RP	CMV-TetR (RFP- P) Lentiviral particles	
LVP017-Bsd	CMV-TetR (Bsd) Lentiviral particles	
LVP017-Neo	CMV-TetR (Neo) Lentiviral particles	
LVP017-Puro	CMV-TetR (Puro) Lentiviral particles	
LVP017-Hygro	CMV-TetR (Hygro) Lentiviral particles	
LVP459-GB	EF1a-TetR (GFP-Bsd) Lentiviral particles	
LVP459-RB	EF1a-TetR (RFP-Bsd) Lentiviral particles	
LVP459-RP	EF1a-TetR (RFP-Puro) Lentiviral particles	
LVP459-Bsd	EF1a-TetR (Bsd) Lentiviral particles	
LVP459-Neo	EF1a-TetR (Neo) Lentiviral particles	
LVP459-Puro	EF1a-TetR (Puro) Lentiviral particles	
LVP459-Hygro	EF1a-TetR (Hygromycin) Lentiviral particles	
LVP017-GB-PBS	CMV-TetR (GFP-Bsd) Lentiviral particles in PBS	200ul, (1 x 10^8 IFU/mL) in PBS
LVP017-RB-PBS	CMV-TetR (RFP- Bsd) Lentiviral particles in PBS	
LVP017-RP-PBS	CMV-TetR (RFP- Puro) Lentiviral particles in PBS	
LVP017-Bsd-PBS	CMV-TetR (Bsd) Lentiviral particles in PBS	
LVP017-Neo-PBS	CMV-TetR (Neo) Lentiviral particles in PBS	
LVP017-Puro-PBS	CMV-TetR (Puro) Lentiviral particles in PBS	

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LVP017-Hygro-PBS	**CMV-TetR (Hygro) Lentiviral particles in PBS**	
LVP459-GB-PBS	**EF1a-TetR (GFP-Bsd) Lentiviral particles in PBS**	
LVP459-RB-PBS	**EF1a-TetR (RFP- Bsd) Lentiviral particles in PBS**	
LVP459-RP-PBS	**EF1a-TetR (RFP-Puro) Lentiviral particles in PBS**	
LVP459-Bsd-PBS	**EF1a-TetR (Bsd) Lentiviral particles in PBS**	
LVP459-Neo-PBS	**EF1a-TetR (Neo) Lentiviral particles in PBS**	
LVP459-Puro-PBS	**EF1a-TetR (Puro) Lentiviral particles in PBS**	
LVP459-Hygro-PBS	**EF1a-TetR (Hygromycin) Lentiviral particles in PBS**	

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

Product Description:

Lentiviral particles or lentivirus is a gene delivery tool produced from lentivectors for gene expression or knockdown. 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 **tetracycline repressor (TetR)** lentiviral particles are generated from GenTarget's re-engineered lentivector system. Sequence fully verified **TetR gene** was constitutively expressed under either a our proprietary super CMV promoter (**suCMV**) or an **enhanced EF1a** promoter. The suCMV promoter provides highest TetR protein levels. The enhanced EF1a promoter is non-tissue specific promoter (active in almost all cell types) and does not be silenced after long term cell culture.

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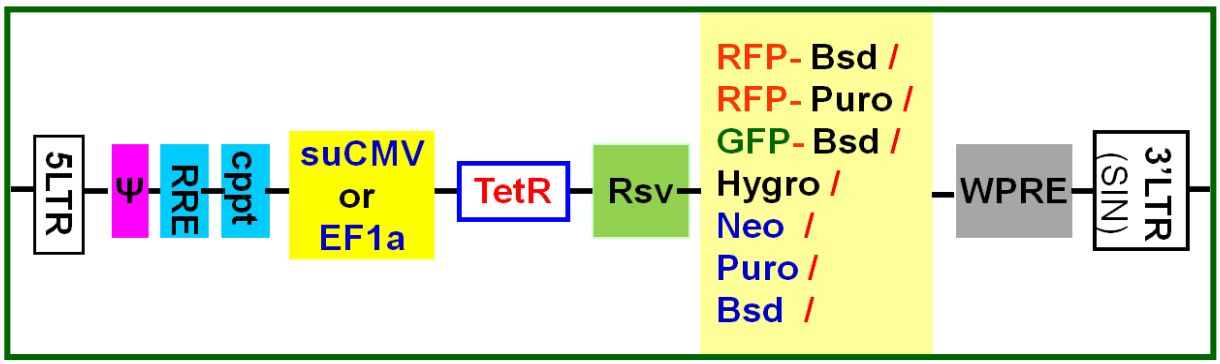
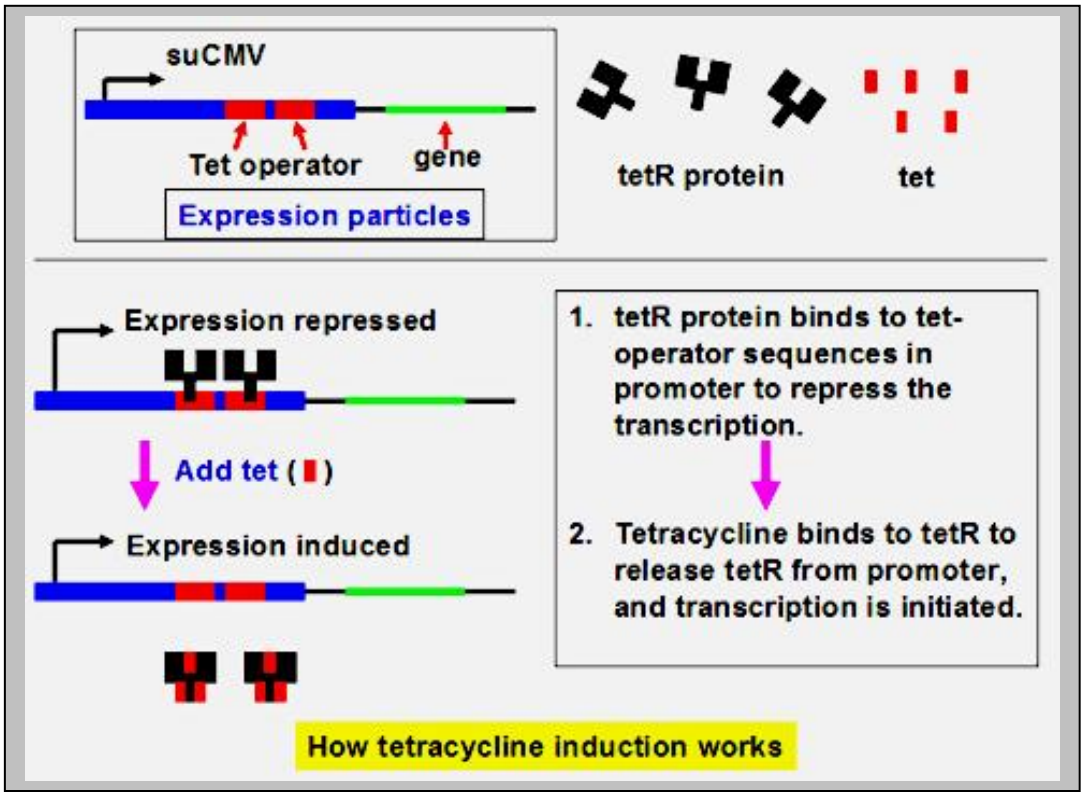


GenTarget provides the TetR expression particles with different antibiotic selection markers or fluorescent-antibiotic fusion dual markers under a separate RSV promoter. Please see the vector map schemes above for the expression lentivector core structure.

TetR is used in tetracycline inducible expression. It binds to any inducible promoters that have incorporated its binding sequence to repress target expression. And target expression is induced once tetracycline is added. The added tetracycline binds to TetR, which releases TetR from target's promoter, and starts the transcription.



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VSV-G pseudotyped lentiviral particles are generated in 293T cell, and provided as 200ul/per vial in **two formats:**

1. packaged in DMEM medium with 10% of FBS with 10x polybrene; or
2. concentrated and re-suspended in PBS solution. The virus in PBS is used for trasnduce the cells that do not want serum and polybrene in the culture medium.

See FAQ for pre-made lentiviral particles (.pdf) for other questions.

Gentarget's **lentiviral inducible expression vectors** contain a strong constitutive promoter (CMV or H1) that integrated with two copies of tetracycline regulator binding sequences. This modification does not change promoter's constitutive expression status. The GOI (gene of interest) can be expressed in high level as regular promoter without any induction. However, **optionally**, Gentarget's lentiviral particles particles can be turned into tetracycline inducible system by using TetR expressing particles. To achieve this inducible expression, the TetR protein has to be present to bind (block) the expression in advance. And the expression is induced after addition of tetracycline which removes the TetR from the promoter. See the illustration figure below about the inducible mechanism.

Please see Optional inducible lentiviral system on our website under Technology for more information.

Key features:

1. High level of TetR expression (driven by a super CMV promoter or an enhanced EF1a), demonstrated minimal basal expression from inducible expression vectors or their expression particles;
2. Deliver TetR expression into divided and non-divided host mammalian cell lines via high virus titers;
3. Different antibiotic selection satisfies the requirement from different inducible expression vectors/ particles (i.e. the double antibiotic selection);
4. Depend upon the cell lines and the TetR expression levels, normally a 20-fold to 1000-fold induction can be obtained after addition of tetracycline;

Applications:

The premade TetR lentiviral particle is the best method to delivery the TetR protein. Gentarget provides TetR expression lentiviral particles with different antibiotic markers. TetR expression particles can be used as follows:

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1. It can be transduced alone into any host cells of your interest to generate TetR expression stable cell line. The generated stable cell is **then** transduced with any inducible target expression particles, and the double transduced cells will demonstrate a tetracycline dose-dependent inducible expression of the target.
2. It can be **co-transduced** with any inducible target expression lentiviral particles. And the double transduced cells (selected via double antibiotic markers) will demonstrate a tetracycline dose-dependent inducible expression of the target.

Protocols (as general reference**)**:

Method A: transduce TetR lentiviral particles alone to generate TetR-stable cell line:

1. plate cells in 0.5 ml of complete medium into each well in 24-well plate, incubated at 37^{o}C for overnight,
2. At the time of transduction, the cell density should be at around 50% confluent. Thaw TetR lentiviral particle, add 20ul ~ 100ul into one well dependent upon cell types (Note: Note: active divided cells have higher transduction efficiency, therefore, less amount virus is needed). Optionally, add polybrene into medium at final concentration of 6ug/ml (Note: polybrene can enhance transduction efficiency, but it may be toxic to some cell lines, like some primary neuron cells.), incubated cells at 37^{o}C for **72 hours,**
3. Remove the medium and replace with fresh, complete medium containing the appropriate amount of antibiotic (dependent TetR particle types) to select for stably transduced cells;
4. Trypsinize cells and pass into new well in 24-well plate in complete medium with appropriate amount of antibiotic (Note: a kill curve may have to be tested first to determine the minimal concentration of the appropriate antibiotic that is required to kill your untransduced cells), and replace medium containing antibiotic every 2-3 days until resistant colonies can be identified (Note: it takes 2-4 weeks dependent upon antibiotic types).
5. Pick several resistant colonies and expand each clone into flask and assay for Tet repressor expression by Western Blot, ELISA or qRT-PCR (Note: TetR assay materials need to be obtained form other vendors and is not provide with TetR particles. **To verify the TetR protein expression by WB, you can use any anti wild-type TetR antibody, such as Boca Scientific's CAT#: TET02, or Clontech's CAT# 631132**). Alternatively, you may pool the heterogeneous population of resistant cells and assay for Tet repressor expression. If Bsd-RFP dual marker TetR particles (Cat#: **LVP017-Bsd-RFP**) are used, the TetR expression cells can be identified via RFP fluorescent under microscope.
6. Positive transduced cells are ready for transfection with inducible expression vectors or transduction with inducible expression lentiviral particles, for the tetracycline inducible expression.
7. At 48 hours after deliver target expression vector/particles, change medium and add tetracycline to induce expression of the target. (Note: vary the final concentration of tetracycline from 0.01 ug/ml to 1 ug/ml to obtain different expression levels of target).

Method B: co-transduce TetR lentiviral particles and expression particles for tetracycline inducible expression:

1. Plate cells in 0.5 ml of complete medium each well in 24-well plate, incubated at 37^{o}C for overnight,
2. At the time of transduction, the cell density should be at around 50% confluent. Thaw TetR lentiviral particles, add 50ul ~ 100ul of TetR particles into one well, dependent upon cell types (Note: active

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divided cells have higher transduction efficiency, therefore, less amount virus is needed). Optionally, add polybrene into medium at final concentration of 6ug/ml (Note: polybrene can enhance transduction efficiency, but it may be toxic to some cell lines, like some primary neuron cells.), incubated cells at 37°C for **24 hours**,

3. Then, add ~50ul of inducible expression particles into the well in 24-well plates (Note: ideally, the TetR particles/ expression particles has the MOI ratio at 5:1, but in general, used TetR particles should more than expression particles), incubated at 37°C for overnight,
4. Add tetracycline to induce expression. The amount of tetracycline to use is dependent upon cell types, a common used final concentration is 1ug/ml. (Note: many bovine sera used in culture are contaminated with tetracycline or its derivatives, which can affect basal expression),
5. Alternatively, at 48~72 hours after both transduction, add antibiotics to select for stably transduced cells (Note: add both antibiotics for TetR particles and expression particles at the same time to select double transdued cells).
6. After obtain the double transduced cells, add tetracycline to induce expression of the gene of interest. (Note: vary the concentration of tetracycline from 0.1 ug/ml to 2 ug/ml to obtain different expression levels of target).

Example / Control for inducible expression:

The following picture demonstrated the GFP expression from premade inducible GFP lentiviral particles (Cat#: LVP024) before and after induction.

Left image: Inducible GFP particles (LVP024) transduced on HEK293 cells;
Middle image: Inducible GFP particles transduced in TetR stable cells;
Right inage: Inducible GFP particles transduced in TetR stable cells and induced by 1ug/ml Tet.

Related products:

Products	Name	Applications
Product series (>500 CAT#)	**Premade expression ready lentiviral particle for human and mouse genes**	• Used as constitutive expression of a human or mouse target. • Or used with TetR particles together for inducible expression of a human or mouse target.

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Inducible expression control viruses	GFP (CAT#:**LVP024**) YFP (CAT#: **LVP357**) RFP (CAT#: **LVP531**)	Control GFP, RFP or YFP lentiviral particles for validation of inducible expression.
TetR expression stable cell lines	Premade TetR Stable cell lines in 293 host cells with different antibiotic selection markers (CAT#: **SC005**) .	Used for tetracycline inducible expression of any constructs with TetR binding sequence in their promoter.

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 when handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

References:

1. Annu Rev Microbiol. 1994;48:345-69.
2. Microbiol Mol Biol Rev. 2005 Jun;69(2):326-56.
3. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors. (Link).
4. CDC guidelines for Lab Biosafety levels (Link).

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

Lentivirus Category (click to see)	**Product Description**
Target Expression	Premade lentivirus express a **human, mouse or rat** gene with Fluorescent-Antibiotic fusion dual selection.
Luciferase expression	Premade lentivirus express all kinds of luciferase**: firefly; Renilla; Cypridina; Red-Luc; Nano-Luc,** with different fluorescent and antibiotic selection.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP/niRFP /unstable GFP, etc.**
Cytoskeleton Imaging	Fluorescent (GFP / RFP/ CFP) labelled cell skeleton protein (Actin; Tubulin; Paxillin; Vimentin)
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent labelled cell organelle lentivirus for living cell imaging.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing by **CRISPR**
Fluorescent Fusion target	Lentivirus express the "**Fluorescent-Target**" fusion proteins. A desired target is fused to Green, Blue, Red, or Cyan

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	Fluorescent Protein, demonstrating the target's functionality and localization
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent 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	**SEAP** (Secreted Embryonic Alkaline Phosphatase) secreted expression lentivirus under different promoter.
TetR repressor expression	Premade lentivirus expressin **TetR** (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	Lentivirus express the reverse tetraccycline transcription activator gene, rtTA-M2 with different selection.
Pathway Reporter	Different Report lentivirus (**Luc, RFP, GFP, SEAP**) under a pathway specific response promoter.
Cell Immortalization	Comprehesive lentivirus for cell immortalization, for different cell types.
Cell Specific reporter	Different Report lentivirus driven by cell specific promoter.
Infectious Antigens	Lentivirus express all kinds of infectious antigens.
Viral Like Particle (VLP)	Lentiviral particles pseudo-typed with high density of surface envelope protein.
Immuno Therapy	Lentivirus products for Immuno Therapy application.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers
LacZ expression	Express different full length **β- galactosidase (lacZ)** with different selection markers
Anti-miNA lentivirus	Pre-made lentivirus expression a specific **anti-miRNA** cassette.
Pre-made shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more).
microRNA and anti-microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
Negative control lentiviruses	Premade **negative control lentivirus with different markers**: serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus

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	target expression effects.
Other Enzyme	Ready-to-use lentivirus, expressing **specific enzymes** with different selection markers.