



## Pre-made tetracycline regulator (TetR) expression lentiviral particles user manual

Cat#	Product Name	Amounts
<a href="#">LVP017-Bsd-RFP</a>	TetR ( <b>Bsd-RFP</b> ) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP017-Bsd</a>	TetR ( <b>Bsd</b> ) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP017-Neo</a>	TetR ( <b>Neo</b> ) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP017-Puro</a>	TetR ( <b>Puro</b> ) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP017-Hygro</a>	TetR ( <b>Hygro</b> ) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP459-(GFP-Bsd)</a>	EF1a-TetR ( <b>GFP-Bsd</b> ) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP459-(Bsd-RFP)</a>	EF1a-TetR ( <b>Bsd-RFP</b> ) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP459-(RFP-Puro)</a>	EF1a-TetR ( <b>RFP-Puro</b> ) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP459-(Bsd)</a>	EF1a-TetR ( <b>Bsd</b> ) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP459-(Neo)</a>	EF1a-TetR ( <b>Neo</b> ) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)
<a href="#">LVP459-(Puro)</a>	EF1a-TetR ( <b>Puro</b> ) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)

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

### **Product Description:**

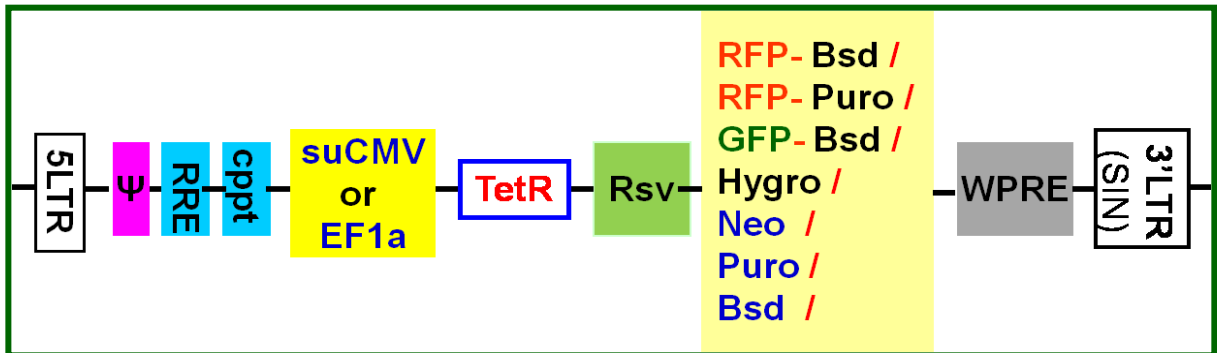
Lentiviral system is a gene delivery tool using lentivectors for gene expression or knockdown. Lentivectors are HIV-1 (Human Immunodeficiency Virus 1) derived plasmids, used to generate lentiviral particles (lentivirus) that can be transduced into almost all kinds of mammalian cell types or organs, including stem cells, primary cells and non-dividing cells both *in vivo* and in **cell culture** system. And particles stably integrate into transduced cells' genome for long term expression. Therefore, lentivirus holds unique promise as gene transfer agents.

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 shows no promoter silencing effects after long term cell culture.

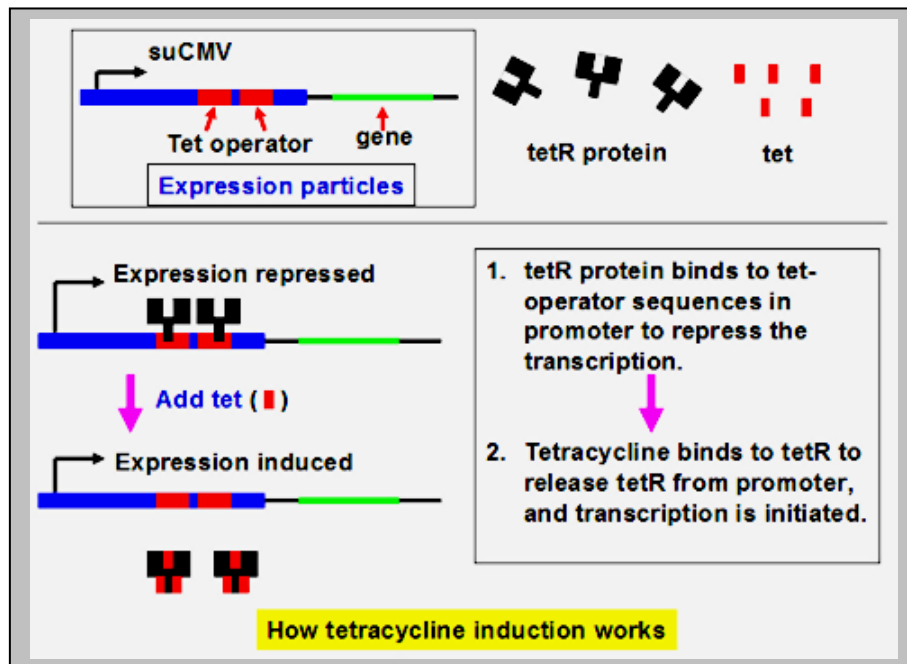


GenTarget provides the TetR expression particles with different antibiotic selection markers or fluorescent-antibiotic fusion dual markers under a separate RSV promoter. Please see the vector map schemes below for the expression lentivector core structure.

VSV-G pseudotyped lentiviral particles are generated in 293T cell, and packaged either in DMEM medium with 10% of FBS, supplied as 200ul/per tube. See [FAQ for pre-made lentiviral particles \(.pdf\)](#) for details.



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.





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 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 [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:

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°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°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 2-5 weeks dependent upon antibiotic types).
5. Pick several resistant colonies and expand each clone into flask and assay for Tet repressor expression by ELISA or qRT-PCR (Note: TetR assay materials need to be obtained from other vendor and is not provided with TetR particles). 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).

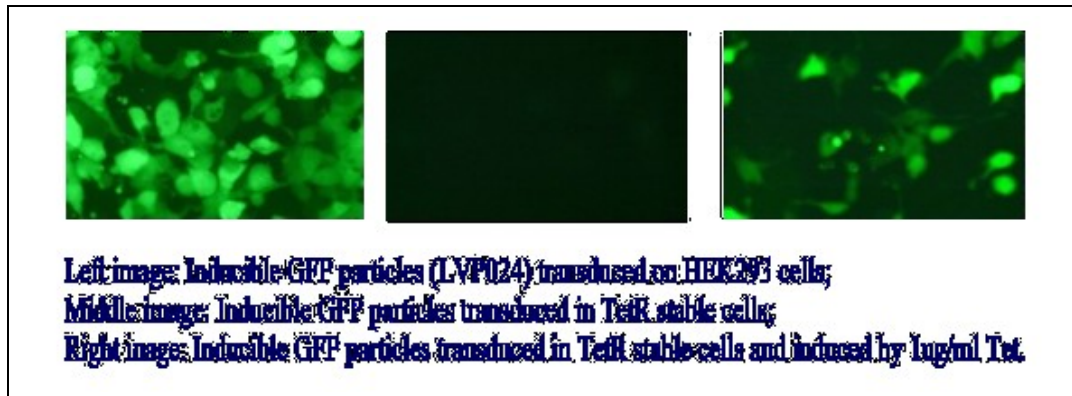
#### **Method B:** co-transduce TetR lentiviral particles and expression particles for tetracycline inducible expression:

1. Plate cells in 0.5 ml of complete medium each well in 24-well plate, incubated at 37°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 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 10 ~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 transduced 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 0.01 ug/ml to 1 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.



## Related products:

Cat#	Name	Applications
<a href="#">Product series, ~300 Catalog Numbers.</a>	<u>Premade expression ready inducible lentiviral particle for human and mouse genes</u>	<ul style="list-style-type: none"><li>• Used as constitutive expression alone,</li><li>• Or paired with TetR lentiviral particles, used as inducible expression,</li></ul>
<a href="#">LVP024</a>	GFP inducible control lentiviral particles	Control GFP lentiviral particles for validation of inducible expression.
<a href="#">SC005</a>	Premade TetR Stable cell lines In 293 host cells	Used as host cell line for premade lentiviral particle product series for inducible expression of human or mouse genes.

## 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.



# GenTarget Inc

6640 Lusk Blvd. Suite A107  
San Diego, CA 92121  
Phone: (858) 6788683  
Fax: (800) 3804198  
Email: [orders@gentarget.com](mailto:orders@gentarget.com)

---

## 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).