



## Pre-made reverse tetracycline Transcriptional Activator (rtTA) Expression Lentiviral Particles

Cat#	Product Name	Amounts
<a href="#">LVP400-RB</a>	rtTA ( <b>RFP-Bsd</b> ) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL)  in DMEM medium containing 10% FBS and 10x polybrene (60ug/ml)
<a href="#">LVP400-GB</a>	rtTA ( <b>GFP-Bsd</b> ) Lentiviral particles	
<a href="#">LVP400-RP</a>	rtTA ( <b>RFP-Puro</b> ) Lentiviral particles	
<a href="#">LVP400-GP</a>	rtTA ( <b>GFP-Puro</b> ) Lentiviral particles	
<a href="#">LVP400-Bsd</a>	rtTA ( <b>Bsd</b> ) Lentiviral particles	
<a href="#">LVP400-Neo</a>	rtTA ( <b>Neo</b> ) Lentiviral particles	
<a href="#">LVP400-Puro</a>	rtTA ( <b>Puro</b> ) Lentiviral particles	
<a href="#">LVP400-RB-PBS</a>	rtTA ( <b>RFP-Bsd</b> ) Lentiviral particles in PBS	200ul, (5 x 10 <sup>7</sup> IFU/mL) in PBS
<a href="#">LVP400-GB-PBS</a>	rtTA ( <b>GFP-Bsd</b> ) Lentiviral particles in PBS	
<a href="#">LVP400-RP-PBS</a>	rtTA ( <b>RFP-Puro</b> ) Lentiviral particles in PBS	
<a href="#">LVP400-GP-PBS</a>	rtTA ( <b>GFP-Puro</b> ) Lentiviral particles in PBS	
<a href="#">LVP400-Bsd-PBS</a>	rtTA ( <b>Bsd</b> ) Lentiviral particles in PBS	
<a href="#">LVP400-Neo-PBS</a>	rtTA ( <b>Neo</b> ) Lentiviral particles in PBS	
<a href="#">LVP400-Puro-PBS</a>	rtTA ( <b>Puro</b> ) Lentiviral particles in PBS	

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

### **Product Description:**

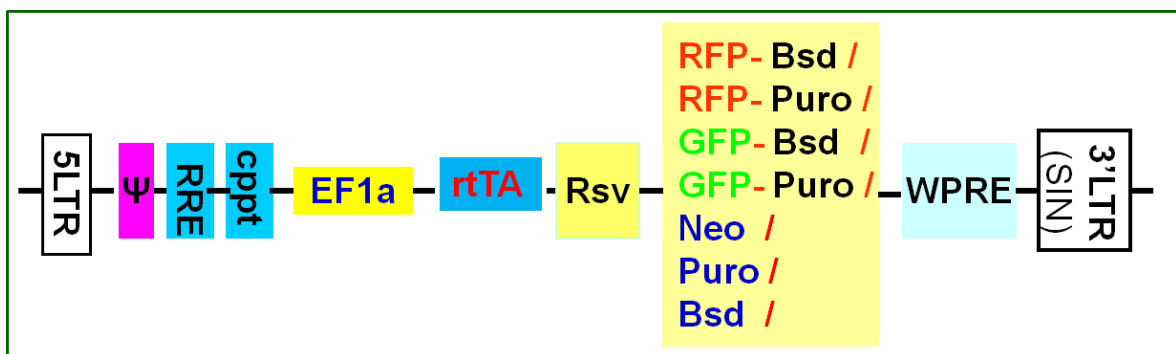
GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into almost all kinds of mammalian cells, including stem cells, primary cells, and non-dividing 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.

The pre-made **reverse tetracycline Transcriptional Activator (rtTA)** lentivirus are generated from GenTarget's re-engineered lentivector system. They constitutively expressed the **rtTA-m2** ([ABC65845.1](#)) gene under an **enhanced EF1a** promoter. 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.

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



**rtTA-M2** is a mutant form of rtTA that has increased stability, reduced background expression, and improved inducibility in the absence of doxycycline (Dox, tetracycline analog).

VSV-G pseudotyped lentiviral particles are generated in 293T cells, and provided as 200 µl/per tube in **two solutions**:

- Packaged in DMEM medium with 10% of FBS with 10x polybrene
- Concentrated into PBS solution for transduction in the absence of serum and polybrene

See [FAQs for pre-made lentiviral particles](#) (.pdf) for more details.

### **Applications:**

rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Transcription can be activated by any promoter embedded with



TetO sequences when both the rtTA protein the inducer (Dox or tetracycline) are present (this is an example of the so-called, **Tet-On** inducible system). The rtTA expression particles can be used either for tetracycline- or Dox-inducible target expression when used with an expression vector containing a TetO inducible promoter or for generation of rtTA expression stable cell lines.

## Key features:

- **High level rtTA expression**, and **minimal basal expression** from inducible Tet-On expression vectors
- rtTA expression in **dividing and non-dividing host mammalian cell lines**
- A wide variety of available **antibiotic selection markers**

## Protocol:

### **Transduction with rtTA lentiviral particles to generate an rtTA-stable cell line:**

1. Plate cells in 0.5 ml of complete medium into each well in a 24-well plate; incubate at 37 °C overnight.
2. Transduction should be carried out when cells are approximately 50% confluent. Thaw rtTA lentiviral particles, add 20-100 µl into each well depending on cell type (Note: actively dividing cells have higher transduction efficiency and require less virus than less rapidly dividing cells). Incubate cells at 37 °C for 72 hours.
3. Remove the medium and replace with fresh, complete medium containing the appropriate amount of antibiotic to select for stably transduced cells.
4. Trypsinize cells and passage into new wells in a 24-well plate in complete medium with an appropriate amount of antibiotic. **Note:** a kill curve may have to be generated first to determine the minimal concentration of antibiotic required to kill untransduced cells.
5. Replace medium containing antibiotic every 2-3 days until resistant colonies can be identified. **Note:** this may take 2-5 weeks depending upon antibiotic.
6. Pick several resistant colonies and expand each clone into a flask. Assay for rtTA expression by Western Blot, ELISA or qRT-PCR. Alternatively, you may pool the heterogeneous population of resistant cells and assay for rtTA expression (**To verify the rtTA protein expression by WB, you can use any anti rtTA antibody, such as Boca Scientific's CAT#: TET02**). If there is a fluorescent marker, the rtTA expressing cells can be sorted or identified via GFP or RFP fluorescence by fluorescence microscopy.



7. Positive rtTA transduced cells are ready for transfection with Tet-On inducible expression vectors (TetOn vectors) for tetracycline / Dox inducible target expression.

## **Safety Precaution:**

GenTarget lentiviral particles adapt 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. PNAS, July 5, 2000, V97(14), 7963-7968
2. BMC Developmental Biology 2010, 10:17
3. NIH Guidelines for [Biosafety Considerations for Research with Lentiviral Vectors](#). (Link).
4. [CDC guidelines for Lab Biosafety levels](#) (Link).

## **Related products: GenTarget's Pre-made lentivirus Products:**

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



<a href="#">LoxP ColorSwitch</a>	Premade lentivirus expressing "LoxP- <b>GFP</b> -Stop-LoxP- <b>RFP</b> " cassette, used to monitor the CRE recombination event in vivo.
<a href="#">SEAP Reporter</a>	<b>SEAP</b> (Secreted Embryonic Alkaline Phosphatase) secreted expression lentivirus under different promoter.
<a href="#">TetR repressor expression</a>	Premade lentivirus expressing <b>TetR</b> (tetracycline regulator) protein, the repressor protein for the inducible expression system.
<a href="#">rtTA Expression</a>	Lentivirus express the reverse tetracycline transcription activator gene, rtTA-M2 with different selection.
<a href="#">Pathway Reporter</a>	Different Report lentivirus ( <b>Luc, RFP, GFP, SEAP</b> ) under a pathway specific response promoter.
<a href="#">Cell Immortalization</a>	Comprehensive lentivirus for cell immortalization, for different cell types.
<a href="#">Cell Specific reporter</a>	Different Report lentivirus driven by cell specific promoter.
<a href="#">Infectious Antigens</a>	Lentivirus express all kinds of infectious antigens.
<a href="#">Viral Like Particle (VLP)</a>	Lentiviral particles pseudo-typed with high density of surface envelope protein.
<a href="#">Immuno Therapy</a>	Lentivirus products for Immuno Therapy application.
<a href="#">iPS factors</a>	Premade lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FGF4</b> ) factors with different fluorescent and antibiotic markers
<a href="#">LacZ expression</a>	Express different full length <b><math>\beta</math>-galactosidase (lacZ)</b> with different selection markers
<a href="#">Anti-miRNA lentivirus</a>	Pre-made lentivirus expression a specific <b>anti-miRNA</b> cassette.
<a href="#">Pre-made shRNA lentivirus</a>	Premade shRNA lentivirus for knockdown a specific genes ( <b>P53, LacZ, Luciferase</b> and more).
<a href="#">microRNA and anti-microRNA lentivirus</a>	Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human and mouse miRNA.
<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentivirus treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme</a>	Ready-to-use lentivirus, expressing <b>specific enzymes</b> with different selection markers.