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

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

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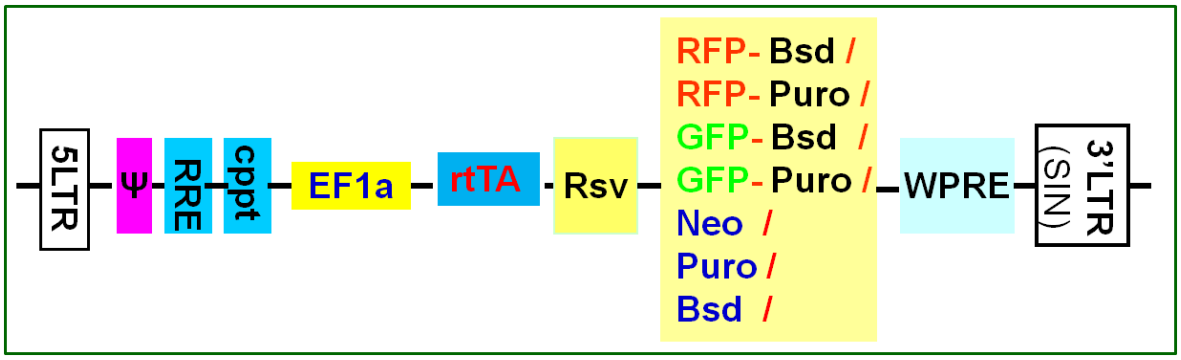
transduced cells' genome for long term expression, making it a great gene transfer agent.

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

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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.

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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 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. Ware glove all the time at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

References:

1. 1. PNAS, July 5, 2000, V97(14), 7963-7968
2. 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).

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/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;
CRISPR Gene Editing	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease, the **dCas9**, gRNAs, **CRISPR** gene editing research

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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	Llentivirus 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 verifeid 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-miNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Human and mouse ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin 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, niRFP, unstable GFP and others.
Luminescent Imaging	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
Sub-cellular Imaging	Lentivirus contain a well-defined organelle targeting signal fusioned 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

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Unstable GFP	Lentivirus express the 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 Lentiviurs 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 flurescent and antibiotic markers.
CRE, Flp ColorSwtich	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 expressin 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	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
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 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.

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