GenTarget Inc

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Validated shRNA Lentivirus for expression knockdown

Cat#	Product Name	Amounts
LVP343-GB; **LVP343-GB-PBS;**	Lentiviral particles, shRNA (**h P53**)-(**GFP-Bsd**)	1 x10^7 IFU/ml x 200ul Or 5 x10^7 IFU/ml x 200ul in PBS
LVP343-GP; **LVP343-GP-PBS;**	Lentiviral particles, shRNA (**h P53**)-(**GFP-Puro**)	
LVP343-RB; **LVP343-RB-PBS;**	Lentiviral particles, shRNA (**h P53**)-(**RFP-Bsd**)	
LVP343-RP; **LVP343-RP-PBS;**	Lentiviral particles, shRNA (**h P53**)-(**RFP-Puro**)	
LVP344-GB; **LVP344-GB-PBS;**	Lentiviral particles, shRNA (**lacZ**)-(**GFP-Bsd**)	
LVP344-GP; **LVP344-GP-PBS;**	Lentiviral particles, shRNA (**lacZ**)-(**GFP-Puro**)	
LVP344-RB; **LVP344-RB-PBS;**	Lentiviral particles, shRNA (**lacZ**)-(**RFP-Bsd**)	
LVP344-RP; **LVP344-RP-PBS;**	Lentiviral particles, shRNA (**lacZ**)-(**RFP-Puro**)	
LVP345-GB; **LVP345-GB-PBS;**	Lentiviral particles, shRNA (**Luc**)-(**GFP-Bsd**)	
LVP345-GP; **LVP345-GP-PBS;**	Lentiviral particles, shRNA (**Luc**)-(**GFP-Puro**)	
LVP345-RB; **LVP345-RB-PBS;**	Lentiviral particles, shRNA (**Luc**)-(**RFP-Bsd**)	
LVP345-RP; **LVP345-RP-PBS;**	Lentiviral particles, shRNA (**Luc**)-(**RFP-Puro**)	
H1(shRNA-Ctr)-GB; **H1(shRNA-Ctr)-GB-PBS**	Lentiviral particles, shRNA (**Neg**)-(**GFP-Bsd**)	
H1(shRNA-Ctr)-GP; **H1(shRNA-Ctr)-GP-PBS;**	Lentiviral particles, shRNA (**Neg**)-(**GFP-Puro**)	
H1(shRNA-Ctr)-RB; **H1(shRNA-Ctr)-RB-PBS;**	Lentiviral particles, shRNA (**Neg**)-(**RFP-Bsd**)	
H1(shRNA-Ctr)-RP; **H1(shRNA-Ctr)-RP-PBS;**	Lentiviral particles, shRNA (**Neg**)-(**RFP-Puro**)	

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

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Product Introduction:

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.

RNA interference (RNAi) technology is a powerful tool for loss-of-function (knockdown/silencing) research in mammalian cells. Originally observed to inhibit gene expression *in vivo* through short double-stranded RNAs, RNAi works through a series of enzymatic reactions mediated by short RNAs having sequences complementary to those of the silenced target. These reactions result in target mRNA degradation or translational repression.

RNAi knockdown can be introduced by short synthetic double-strand RNA (siRNA) or by vector-expressed stem-hairpin RNA (shRNA) which is further processed by Dicer enzyme to produce double-strand short RNAs. Chemically synthesized double stranded RNA (siRNA) has a transient silencing effect only; in contrast, selection of clones for stable vector-expression of RNAi can provide long term silencing.

GenTarget's Lentiviral shRNA Expression System:

GenTarget has designed and constructed a set of lentiviral shRNA expression cloning kits (click to see product page). The target specific shRNA is expressed under the constitutive human U6 promoter, or under an optional inducible human H1 promoter. This H1 promoter allows you to choose between constitutive and tetracycline inducible expression of shRNA**.** Please refer to our website for more details about the optional inducible expression mechanism**.**

This optional inducible knockdown (for H1 promoter only) requires the TetR must be expressed in advance or at the same time as shRNA transduction. The presence of TetR can be achieved by the following methods:

- **TetR stable cell lines** that constitutively express the TetR protein
- **Co-transfection** with a TetR expression plasmid and a target-inducible expression vector

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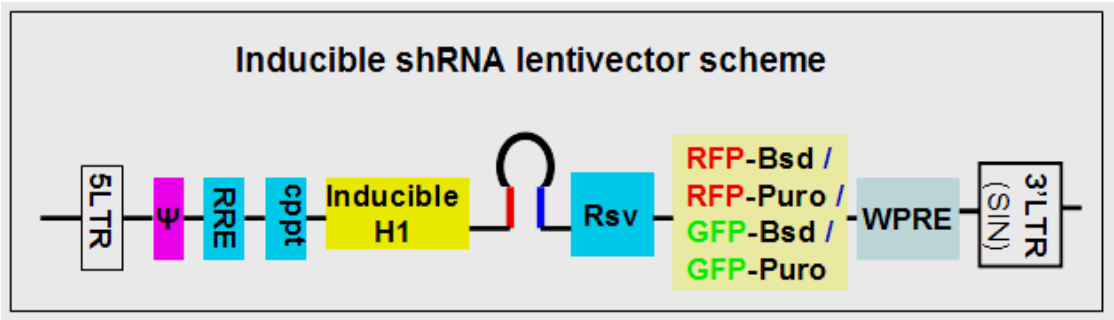
- **Co-transduction** with TetR lentiviral particles and inducible gene expression lentiviral particles. Double antibiotic selection is used for co-transduced cells

GenTarget provides "**premade tetR particles**" with a variety of antibiotics for double selection of transduced cells.

Each shRNA lentivirus contains an antibiotic marker or a "fluorescent - antibiotic" fusion dual marker under constitutive RSV promoter. These markers provide a convenient method for real-time monitoring of shRNA expression and viral transduction efficiency by fluorescence and antibiotic selection of stable shRNA positive cells. (Note: RSV promoter strength in your assay cell types determines the fluorescent marker's signal level, but not the knockdown level). See the vector's core structure scheme below.



Validated shRNA lentiviral particles:

The validated shRNA expression particles contain a target specific shRNA hairpin insert (see the **shRNA insert sequence table** below for details) that demonstrates greater than 75-95% knockdown of the target. Knockdown validation was measured via a reporter assay where the specific target was fusioned with a lacZ or luciferase reporter; the knockdown levels were reflected by the decreases of lacZ or luciferase activity. **All validated shRNA are guaranteed greater than 75% knockdown level at the specific endogenous target.**

The premade shRNA lentiviral particles are produced by co-transfection of shRNA lentivector and packaging plasmid into 293T cells. The VSV-G pseudotyped lentiviral particles are provided in 200ul aliquots in DMEM medium, or in PBS solution. For more details about premade particles, please see FAQs for pre-made lentiviral particles (.pdf).

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Simply add the premade shRNA lentivirus into your cell culture, 3 days later, the transduced cells can be selected via antibiotic or via GFP /RFP fluorescent cell sorting, to generate target knockdown cell line. A designed negative control sequence is cloned in the same shRNA lentivector backbone The shRNA-control virus (**shRNA-Ctr**) serves as non-specific knockdown controls for lentivirus treatment.

Note: For your desired target specific shRNA knockdown lentivirus, GenTarget provides shRNA lentivector cloning services. We have the best prices and fast-around times in the industry (see our website for more details).

Key features:

- **High shRNA expression level and validated knockdown**
- **Optional inducible shRNA expression**: particles can be used for constitutive expression knockdown or, optionally, for tetracycline inducible knockdown.
- **Safe to use:** self-inactivation prevents replication of the viron
- **Dual selection**: transduced cells can be sorted via fluorescence or selected for resistance to puromycin or blasticidin
- **Easy to use:** directly add into cultured cells. There is no need for lipids or transfection reagents. Simply add 50 µl into your cell culture in a 24-well plate. (Note: depending upon your specific needs, you may transduce at different MOIs for different levels of expression.)

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shRNA insert sequence table		
Catalog Number	**shRNA hairpin insert (SENSE-loop-ANTISENSE)**	**Product description**
LVP343-GB **LVP343-GP** **LVP343-RB** **LVP343-RP**	GTAATCTACTGGGACGGAACAcgag TGTTCCGTCCCAGTAGATTAC	**h P53 shRNA expression Particles** specifically silence the human P53 gene (NM_000546) with a knockdown level greater than 75% A549 cell via enzymtic validation analysis for exogenous P53 and via Q-RT-PCR analysis for endogenous P53.
LVP344-GB **LVP344-GP** **LVP344-RB** **LVP344-RP**	GACTACACAAATCAGCGATTTcgag AAATCGCTGATTTGTGTAGTC	**LacZ shRNA expression Particles** specifically silence β-Galactosidase (lacZ) gene with a knockdown level greater than 90% in HEK293 cells for endogenous lacZ via enzymtic validation analysis. They can serve as knockdown **postive controls**.
LVP345-GB **LVP345-GP** **LVP345-RB** **LVP345-RP**	GAAACGATATGGGCTGAATACcgag GTATTCAGCCCATATCGTTTC	**Luciferease shRNA expression Particles** specifically silence the firefly luciferase gene with a knockdown level greater than 75% in HEK293 cells for endogenous luciferase expression via enzymtic validation analysis. They can serve as knockdown **postive controls.**
LVP-Ctr-GB **LVP-Ctr--GP** **LVP-Ctr--RB** **LVP-Ctr--RP**	GTCTCCACGCGCAGTACATTTcgag AAATGTACTGCGCGTGGAGAC	**Negative shRNA controls** containing a insert that designed has no homogous to any human or mouse transcripts (should not target any known human or mouse genes). These controls serve as a useful reference for interpretation of knockdown results.

Transduction Protocols:

Note: Pre-made lentivirus is provided ready to use, so it can be simply added into your cell culture; the amount of virus to add depends on cell type. For quick transduction, add 50 µl of virus into each well of 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. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection.

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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 x 10^5/ml x 0.5ml 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, CO_2 incubator.

  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 ^oC for future use; virus titer will decrease by ~10% for each freeze/thaw cycle.

Day 3:

At ~72hr after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava). Then, measure the knockdown level by Q-RT-PCR or WB.

Day 3 (optional**)**:

Sort transduced cells by FACS, and select for antibiotic resistance. 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). Then, measure the knockdown level by Q-RT-PCR or WB on the selected cells.

Note: Filter wavelength settings:
GFP filter: ~Ex450-490 ~Em525;
RFP filter: ~Ex545 ~Em620;

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.

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References:

1. Molecular Therapy (2003) 7, 460–466; doi: 10.1016/S1525-0016(03)00024-8
2. Annu Rev Microbiol. 1994;48:345-69.
3. Microbiol Mol Biol Rev. 2005 Jun;69(2):326-56.
4. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors. (Link).
5. CDC guidelines for Lab Biosafety levels (Link).

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.

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
Epigenomic: CRISPRi and CRISPRa	"**dCas9-Protein**" fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).

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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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Product Category	Product Description (please click into each category's page)
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.