



Pre-made Lentiviral Particles for shRNA expression

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

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

Product Description:

Introduction:

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 virtually all kinds of mammalian cell types or organs, including stem cells,

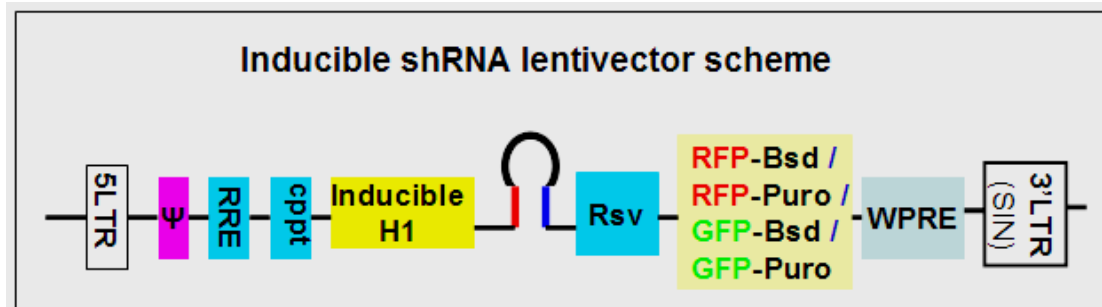


primary cells and non-dividing cells both *in vivo* and in **cell culture** system. Particles stably integrate into the transduced cells' genome for long term expression. Therefore, lentivirus holds unique promise as gene transfer agents.

RNA interference (RNAi) technology is a tool for lost-of-function (knockdown / silencing) studies in mammalian cells. Originally, double-strand short RNAs were found *in vivo*, inhibiting gene expression. The mechanism is a series of enzymatic reactions mediated by short RNAs that complementary in sequence to the silenced targets, leading to mRNA degradation or translational repression. RNAi knockdown can be introduced by synthetic short double-strand RNA (siRNA) or vector expressed stem-hairpin RNA (shRNA) which was further processed by Dicer enzyme to producing double-strand short RNAs. Chemical synthesized double stranded RNA (siRNA) is only for transient silencing effect. In contrast, vector expressed RNAi can provide a long term effect by stable selection.

GenTarget's Lentiviral shRNA expression system:

Vector expressed RNAi (shRNA) provides a convenient method to functional studies in both animal and cell line models. Variety shRNA vectors are commercially available. GenTarget designed and constructed a set of inducible **lentiviral shRNA expression cloning kits** with different selection marker. (Cat#: [LTSH-GB](#), [LTSH-GP](#), [LTSH-RB](#), [LTSH-RP](#)). Each vector has a fusion marker of "fluorescent protein-antibiotic", providing a realtime monitoring method for the shRNA expression (virus transduction efficiency) via the fluorescent signal, and also a method for selecting the shRNA positive stable cells via antibiotic selection (see vector map scheme below).



The shRNA was expressed under an optional inducible human H1 promoter. In other words, the shRNA can be used for constitutive expression without any induction. However, optionally, it can be used as tetracycline inducible expression. For inducible expression, the shRNA expression was repressed in the presence of TetR and induced by addition of tetracycline. If inducible shRNA expression is desirable, the repressor regulator (tetR) expression must be



delivered in advance or at the same time with shRNA transduction. The presence of tetR can be achieved by the following methods:

- tetR is already expressed in a stable cell line that constantly express tetR protein in advance;
- Transfect a tetR expression plasmid before transduce lentiviral particles;
- Co-transduce both the tetR repressor particles and the gene expression particles into the sample cells. The double transduced cells can be selected by double antibiotics, and then used for inducible expression. Gentarget provides “[premade tetR particles](#)” with different antibiotics for double selecting the transduced cells.

(Please refer to our website for more details about [Optional inducible expression mechanism](#)).

Validated shRNA lentiviral particles:

The validated shRNA expression particles contains a target specific shRNA hairpin insert (see the **shRNA insert sequence table** below for details) that demonstrated greater than 75~95% knockdown level to the specific target. The knockdown validation was measured via a reporter assay where the specific target was fused with lacZ or luciferase reporter. The knockdown levels were reflected by the decreases of lacZ or luciferase activity.

The premade shRNA lentiviral particles were produced by co-transfection of shRNA lentivector with packaging plasmids into 293T cells. The VSV-G pseudotyped lentiviral particles were collected in DMEM medium with 10% FBS (no any other additives). For more details about premade particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf). GenTarget also provides [lentiviral shRNA vector cloning and virus production services](#) for your specific shRNA sequences with the best price and fast-around time (see our website for more).

Key features:

1. **High shRNA expression level and validated knockdown;**
2. **Optional inducible shRNA expression:** particles can be used as constitutive expression, or optionally as tetracycline inducible expression;
3. **Safe to use:** Self-inactivation to prevent replication of the viron;
4. **Dual selection:** transduced cells can be sorted via a fluorescent signal or selected via an antibiotics, puromycin or blasticidin;
5. **Easy to use:** directly added into cells. No need any lipids or transfection reagents; Or simply add 50ul into your cell culture in 24-well plate. (Note: dependent upon your specific needs, you may design the transduction with different MOI for different levels of expression.)



shRNA insert sequence table		
Catalog Number	shRNA hairpin insert (SENSE-loop-ANTISENSE)	Product description
LVP343-GB	GTAATCTACTGGGACGGAACcgag 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.
LVP343-GP		
LVP343-RB		
LVP343-RP		
LVP344-GB	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 .
LVP344-GP		
LVP344-RB		
LVP344-RP		
LVP345-GB	GAAACGATATGGGCTGAATACcgag GTATTCAGCCCATATCGTTTC	Luciferase 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 .
LVP345-GP		
LVP345-RB		
LVP345-RP		
LVP-Ctr-GB	GTCTCCACGCGCAGTACATTTcgag AAATGTACTGCGGTGGAGAC	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.
LVP-Ctr--GP		
LVP-Ctr--RB		
LVP-Ctr--RP		

Transduction Protocols (for reference only):

1. Adhesive cells Transduction Protocols:

Day 0: Seed the desired cells in complete medium at appropriate density incubate overnight. (Note: at the time of transduction, it grows to 25% ~50% confluent.)

For example, seed Hela cells at 0.5×10^5 /ml x 0.5ml in a well of a 24-well plate;

Day 1: Thaw the Pre-made lentiviral stock at room temperature. Add appropriate amount of virus stock to obtain the desired MOI. Or simply add 50 ul of virus into one well in 24-well plate without worry about the MOI number. Return cells to 37°C/CO2 incubator. **A common used MOI number is 10.** (Note: add polybrene to final concentration at 6-8 ug/ml may help the transduction to some cell lines.)



Day 3: At the time of ~72hr after transduction, Check the transduction rate via fluorescence image with a suitable filter under fluorescent Microscope, or calculate the exact transduction % rate via Flow Cytometry System (FACS) or any flow cytometry (such as Quava machine). (**Note:** some cell lines need longer time, up to one week to see the transduction effects / the fluorescent signal.)

Day 3 + (optional): Transduced cell can be sorted out via FACS. Or you can select transduced stable cell line by a specific antibiotic (dependent upon the used particles types). A pilot experiment should be done to determine the antibiotic kill curve for your specific cell line.

2. Suspension cells transduction Protocols:

1. Grow your cell in your completed suspension culture medium, shaking in flask in CO2 incubator;
2. Measure cell density. When cell grow to $\sim 3 \times 10^6$ cell/ml, measure cell viability (should > 90%), then diluted cells into 1×10^6 cell/ml in completed medium;
3. Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particle into the diluted cells at ratio of: **200ul virus per 2ml cells** (Note: depend upon the cell types; you may need to use more or less viruses). Grow cells in flask, shaking in CO2 incubator.
4. At 24 hour after transduction, add equal amount of fresh medium containing final concentration of Blastcidin at 5 ~ 10ug/ml depend upon cell types (or other antibiotics dependent upon the particle types). Grow cell shaking in CO2 incubator.
5. At 72 hours after transduction, check fluorescence under microscope or calculate the transduction efficiency using cell sorting machine (like FACS or Guava machine).

(**Note:** GFP filter wavelength: Ex450-490 ~Em525; RFP filter: ~Ex545/~Em620).

Safety Precaution:

Please use extra caution when using lentiviral particles. Remember. Ware glove all the time at handling Lentiviral particles! Please refer CDC and NIH's links (see references) for more details regarding to safety issues.

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 warranted to meet its quality as described when used 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.

These products are for research use only!