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:

Lentiviral particles or lentivirus is a gene delivery tool produced from lentivectors for gene expression or knockdown. AMSBIO's lentivector system are 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 cell's 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.

Lentiviral shRNA Expression System:

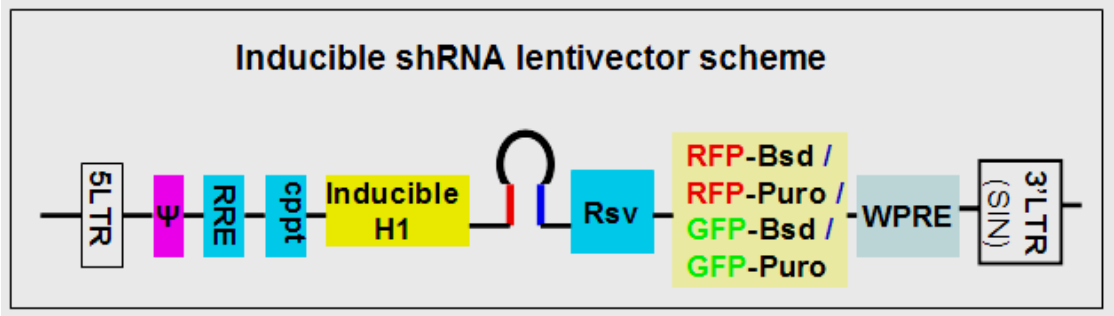
AMSBIO has designed and constructed a set of lentiviral shRNA expression cloning kits. The target specific shRNA is expressed under the constitutive human U6 promoter, or under an optional inducible human H1 promoter. This H1 promoter allows you to choose between constitutive and tetracycline inducible expression of shRNA**.** Please refer to our website for more details about the optional inducible expression mechanism**:** http://www.amsbio.com/Lentivirus.aspx#constitutive

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

AMSBIO provides TetR lentiviral 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 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 to give greater than 75% knockdown level for 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 200 µl aliquots in DMEM medium, or in PBS solution. For more details about premade particles, please see FAQs for pre-made lentiviral particles: pdf.

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 other target specific shRNA knockdown lentivirus, AMSBIO provides shRNA lentivector cloning services.

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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.)

shRNA insert sequence table		
Catalog Number	**shRNA hairpin insert (SENSE-loop-ANTISENSE)**	**Product description**
LVP343-GB	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.
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	**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.**
LVP345-GP		
LVP345-RB		
LVP345-RP		
LVP-Ctr-GB	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.
LVP-Ctr--GP		
LVP-Ctr--RB		
LVP-Ctr--RP		

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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, passage cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection.

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:

- Remove the culture medium and add 0.5ml fresh, warm, complete medium.
- 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°C 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). You can now start treatment of cells for signal pathway assay.

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 literature on generation of stable cell lines).

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

Safety Precaution:

AMSBIO lentiviral particles have adopted the most 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 gloves at all times when handling lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

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References:

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7. Molecular & Biochemical Parasitology 155 (2007) 167–171;
8. Biosci. Biotechnol. Biochem., 68(3), 565-570, 2004;
9. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors link
10. CDC guidelines for Lab Biosafety levels link

Warranty:

This product is warranted to meet its quality as described when used accordance with its instructions. Amsbio disclaims any implied warranty of this product for particular application. In no event shall Amsbio be liable for any incidental or consequential damages in connection with the products. Amsbio's sole remedy for breach of this warranty should be, at Amsbio's option, to replace the products.

These products are provided for research use only!

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