



Validated Knockdown shRNA Lentivirus for human P53

Cat#	Product Name	Amounts
LVP343-GB-PBS	Knockdown Lentivirus, shRNA (h P53) / (GFP-Bsd)	200ul x(1 x10 ⁸ IFU/ml)
LVP343-GP-PBS	Knockdown Lentivirus, shRNA (h P53) / (GFP-Puro)	
LVP343-RB-PBS	Knockdown Lentivirus, shRNA (h P53) / (RFP-Bsd)	
LVP343-RP-PBS	Knockdown Lentivirus, shRNA (h P53) / (RFP-Puro)	
H1(shRNA-Ctr)-GB-PBS	shRNA-H1 (Neg) / (GFP-Bsd) lentivirus	200ul x(1 x10 ⁸ IFU/ml)
H1(shRNA-Ctr)-GP-PBS	shRNA-H1 (Neg) / (GFP-Puro) lentivirus	
H1(shRNA-Ctr)-RB-PBS	shRNA-H1 (Neg) / (RFP-Bsd) lentivirus	
H1(shRNA-Ctr)-RP-PBS	shRNA-H1 (Neg) / (RFP-Puro) lentivirus	
Validated human P53 shRNA sequence: GTAATCTACTGGGACGGAACA		

Storage: -80 °C, avoid repeat freeze/thaw cycles. Stable for 12 months.

1. 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



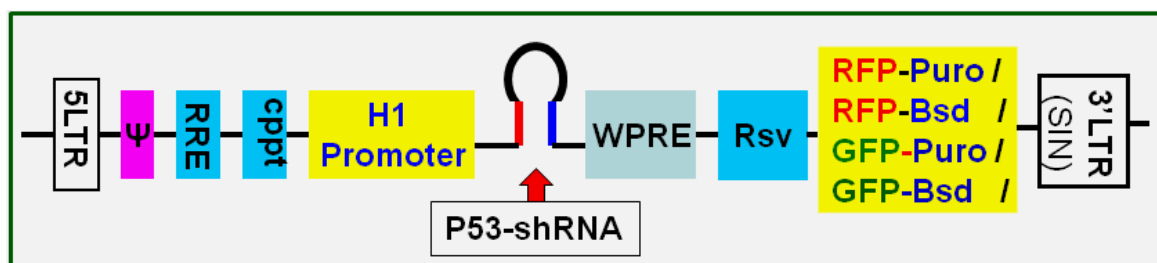
having sequences complementary to those of the silenced target. These reactions result in target mRNA degradation or translational repression.

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.

One Cell immortalization method is to repress the genes that control cell cycle such as such as retinoblastoma (Rb) and p53 genes (the tumor suppressor proteins). Therefore, knockdown human P53 lentivirus is used for cell immortalization for a wide variety of cell types.

2. GenTarget's shRNA Lentivectors:

GenTarget offers the shRNA Lentivirus that knockdown human P53 gene. These pre-made knockdown lentivirus are validated with greater than 75% knockdown level (80% to 97% depends upon cell types). Each P53 shRNA lentivirus contains a **Fluorescent-Antibiotic fusion dual marker** so you can either select the transduced cells by antibiotic killing or sort them via Flow Cytometer. Those products are available in four different dual markers versions as: **GFP-Blasticidin**, **GFP-Puromycin**, **RFP-Blasticidin** and **RFP-Puromycin**. The P53 target specific shRNA is expressed under the human H1 promoter. The fusion dual marker is expressed under RSV promoter. See shRNA lentivector core structure scheme below.



3. Validated shRNA knockdown:

The validated shRNA knockdown contain a human P53 specific shRNA hairpin insert that demonstrates greater than 75-95% knockdown level depends upon cell types. Knockdown validation was measured via a reporter assay



where the specific target was fused with a lacZ or luciferase reporter; the knockdown levels were reflected by the decreases of lacZ or luciferase activity. All validated shRNAs are guaranteed with greater than 75% knockdown level for human P53 endogenous gene.

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).

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 corresponding shRNA-control lentivirus (**shRNA-Ctr**) transcribed a scrambled sequence, having the same selection marker, serves as non-specific knockdown controls for lentivirus treatment.

4. Key features:

- 1) **Validated high knockdown level on human P53 gene;**
- 2) **Safe to use:** self-inactivation prevents replication of the viron;
- 3) **Dual selection:** transduced cells can be sorted via fluorescent signal or selected via Puromycin or Blasticidin killing;
- 4) **Easy to use:** directly add into cultured cells. There is no need for lipids or transfection reagents.

5. Transduction Protocols:

Note: Pre-made lentivirus is provided ready to use status, simply added into your cell culture. The amount of lentivirus to add depends on cell type. In general, add 50 μ l of virus into one well in 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 if applicable. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection. (**Note:** for suspension cells or the "hard-to-transduced" cell type, you may need to double the virus amount added.)



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 $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$ 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, CO₂ incubator. Do nothing.

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 48hr~72hr (Depend upon cell type) after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava).

Day 3 + (optional):

Sort transduced cells by FACS, or select by antibiotic killing. 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).

Note: Filter wavelength settings:

GFP filter: ~Ex450-490 ~Em525;
RFP filter: ~Ex558 ~Em583;

6. 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.

7. 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 \(Link\).](#)

8. 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.

9. **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 humanized 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).
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	Lentivirus that express all kinds of infectious antigens with C-term 6His-tag.



Product Category	Product Description (please click into each category's page)
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 verified 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-miRNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Human and mouse ORFs	Premade lentivirus express in a human, mouse or rat gene with RFP-Blasticidin 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 fused 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
Unstable GFP	Lentivirus express 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 Lentiviruses provides the whole-body images with better contrast and brighter images



Product Category	Product Description (please click into each category's page)
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 fluorescent and antibiotic markers.
CRE, Flp ColorSwitch	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, FGF4) factors with different fluorescent and antibiotic 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 lentiviruses 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.