GenTarget Inc

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Validated Knockdown human P53 shRNA Lentivirus

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**)	
Validated human P53 shRNA sequence: GTAATCTACTGGGACGGAACA		

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

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.

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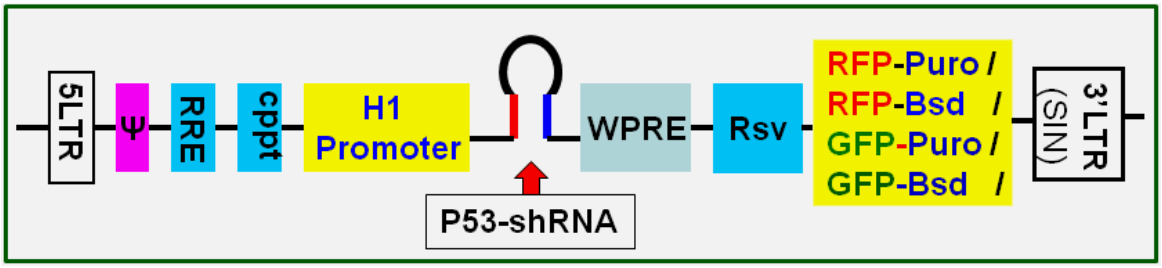
One Cell Immortalization method is to repress the genes that control cell cycle such as such as retinoblastoma (Rb) and p53 genes (the tumor suppressor proteins). Therefore, knockdown human P53 lentivirus is used for cell immortalization for a wide variety of cell types.

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.



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 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 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).

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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.

Key features:

- **High shRNA expression level and validated 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.)

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.

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:

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- 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). 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.

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.

Related Products: GenTarget's pre-made lentivirus product categories:

Lentivirus Category (click to see)	**Product Description**
Target Expression	Premade lentivirus express a **human, mouse or rat** gene with Fluorescent-Antibiotic fusion dual selection.
Luciferase expression	Premade lentivirus express all kinds of luciferase**: firefly; Renilla; Cypridina; Red-Luc; Nano-Luc,** with different fluorescent and antibiotic selection.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP/niRFP /unstable GFP, etc.**
Cytoskeleton Imaging	Fluorescent (GFP / RFP/ CFP) labelled cell skeleton protein (Actin; Tubulin; Paxillin; Vimentin)
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent labelled cell organelle lentivirus for living cell imaging.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing by **CRISPR**
Fluorescent Fusion target	Lentivirus express the "**Fluorescent-Target**" fusion proteins. A desired target is fused to Green, Blue, Red, or Cyan Fluorescent Protein, demonstrating the target's functionality and localization
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.
LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
SEAP Reporter	**SEAP** (Secreted Embryonic Alkaline Phosphatase) secreted expression lentivirus under different promoter.
TetR repressor expression	Premade lentivirus expressin **TetR** (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	Lentivirus express the reverse tetraccycline transcription activator gene, rtTA-M2 with different selection.

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Pathway Reporter	Different Report lentivirus (**Luc, RFP, GFP, SEAP**) under a pathway specific response promoter.
Cell Immortalization	Comprehesive lentivirus for cell immortalization, for different cell types.
Cell Specific reporter	Different Report lentivirus driven by cell specific promoter.
Infectious Antigens	Lentivirus express all kinds of infectious antigens.
Viral Like Particle (VLP)	Lentiviral particles pseudo-typed with high density of surface envelope protein.
Immuno Therapy	Lentivirus products for Immuno Therapy application.
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
Anti-miNA lentivirus	Pre-made lentivirus expression a specific **anti-miRNA** cassette.
Pre-made shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more).
microRNA and anti-microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
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	Ready-to-use lentivirus, expressing **specific enzymes** with different selection markers.