GenTarget Inc

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Pre-made anti-microRNA (for human and mouse) lentivirus User Manual

Catalog#	Product Name	Amounts
A-miRNA H1-Bsd	anti miRNA (H1) with Blasticidin marker	1. 0.5 ml of anti a specific microRNA lentivirus; 2. 0.5ml of Negative control anti-microRNA lentivirus
A-miRNA H1-GB	anti miRNA (H1) with GFP-Blasticidin marker	
A-miRNA H1-GP	anti miRNA (H1) with GFP-Puromycin marker	
A-miRNA H1-Puro	anti miRNA (H1) with Puromycin marker	
A-miRNA H1-RB	anti miRNA (H1) with RFP-Blasticidin marker	
A-miRNA H1-RP	anti miRNA (H1) with RFP-Puromycin marker	
A-miRNA U6-Bsd	Anti miRNA (U6) with Blasticidin marker	
A-miRNA U6-GB	Anti miRNA (U6) with GFP-Blasticidin marker	
A-miRNA U6-GP	Anti miRNA (U6) with GFP-Puromycin marker	
A-miRNA U6-Puro	Anti miRNA (U6) with Puromycin marker	
A-miRNA U6-RB	Anti miRNA (U6) with RFP-Blasticidin marker	
A-miRNA U6-RP	Anti miRNA (U6) with RFP-Puromycin marker	

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

Product Description:

1. Introduction:

MicroRNAs (**miRNAs**) are small non-coding RNA molecule found in plants and animals. They are transcribed from RNA precursors (**pre-miRNAs**), and have a mature length of 19-26 nucleotides. MiRNAs silence or repress gene expression by binding to complementary sequences within gene-coding mRNAs. Each miRNA can target multiple genes, and it is believed that >60% of mammalian gene expression is regulated by miRNAs. This includes most biological processes, including a variety of diseases and developmental disorders. More than 2000 mature miRNAs have been discovered in human and more than 1200 in mouse so far.

Anti-miRNAs, or miRNA inhibitors are short RNA molecules that are complementary to mature miRNAs. Anti-miRNA specifically binds to endogenous miRNA, thereby inhibiting or down-regulating miRNA activity. For this reason, anti-miRNAs are very useful tools for loss-of-function research involving specific miRNAs. Anti-miRNAs are also potential therapeutic agents for some diseases.

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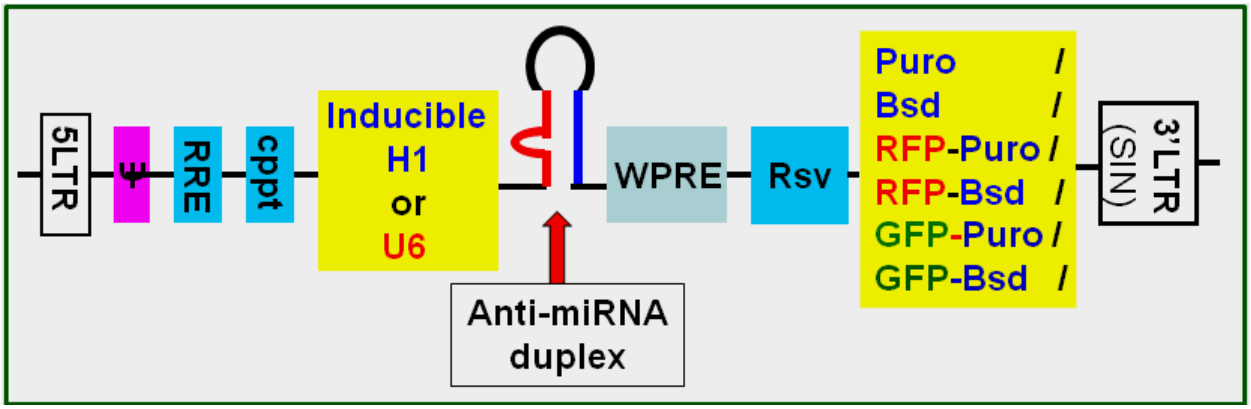
Anti-miRNA can be delivered by two methods: 1) single stranded nucleic acids designed to specifically bind the target miRNA sequence, or 2) vector-based expression of the antisense sequence.

GenTarget's ready-to-use, anti-miRNA lentiviruses are produced from our optimally designed anti-miRNA lentivectors. The annealed DNA oligonucleotide hairpin duplex encoding the antisense sequence of a specific miRNA was cloned under a **constitutive** human U6 promoter or under an **optional inducible** human H1 promoter. GenTarget's anti-miRNA lentivectors have been carefully designed for the highest anti-miRNA expression level, precise promoter transcription position, and favorability of the antisense strand for RISC complex processing. The engineered asymmetric duplex transcribes a perfectly matched antisense RNA molecule and a leftover bulged-out sense RNA molecule. The antisense RNA molecule is expressed at a high level and complementarily "binds" to and inactivates endogenous, mature miRNA. Please see the map below for the anti-miRNA lentivector core structure.

anti-miRNA lentivector core structure:



2. **Pre-made anti-miRNA lentivirus**:

Anti-miRNA lentivirus is the easiest and most reliable method for consistent delivery of anti-miRNA molecules. GenTarget's anti-miRNA lentiviruses feature **a choice of two promoters** (H1 or U6) and **a variety of selection markers** (antibiotic markers or fluorescent-antibiotic fusion markers), providing you with tools for long-term constitutive or inducible inhibition of targeted miRNA functionality. Lentivirus can be transduced into the majority of mammalian cell types, and the human H1 and U6 promoters are active in almost all mammalian cells. GenTarget provides packaged anti-miRNA lentivirus for any specific miRNA from human, mouse, or any other species.

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Click here to see GenTarget's **anti-human miRNA list** and **anti-mouse miRNA list**.

All lentiviruses demonstrate strong transduction efficiency, and each lot of virus is validated for titer of approximately $1x10^{7-8}$ IFU/ml. Quality is guaranteed.

Pre-made lentivirus is provided as ready-to-use **0.5ml** aliquots in DMEM medium with 10% FBS and 60µg/ml polybrene. Upon request, lentivirus can be provided in PBS solution ***in vivo*** use.

For general questions about our ready-to-use lentiviral particles, please see **FAQ for pre-made lentiviral particles** (.pdf) on our website.

Note: GenTarget also provides pre-miRNA expression lentivirus for any specific miRNA; please contact us for a service quote.

3. What is the negative control for anti-miRNA lentivirus?

While each anti-miRNA encodes a specific antisense RNA transcript, a negative control anti-miRNA (**a-miRNA-Neg-control**) lentivirus encodes a designed control sequence with minimal non-specific effects.

4. Why use GenTarget's anti-miRNA lentivirus:

- **The strongest and most stable anti-miRNA expression.** Lentivirus can be effectively transduced into most dividing and non-dividing cells, and anti-miRNA can integrate into the host cell's genome for stable, long-term expression.
- **Compatibility.** The human H1 and human U6 promoters are active in almost all types of mammalian cells.
- **Constitutive or inducible expression.** GenTarget's lentivirus can be used for constitutive high shRNA expression, or optionally, for tetracycline inducible shRNA expression (with the H1 promoter only).
- **Full coverage.** You can order any anti-miRNAs listed in **miRBase** from any species; simply provide us the miRNA ID or the mature sequence, or your own validated anti-miRNA sequences.
- **Options for antibiotic markers.** Select from puromycin, blasticidin, or dual markers such as GFP-puromycin, GFP-blasticidin, RFP-puromycin, RFP-blasticidin.

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Transduction Protocols:

1) Transduction Protocol for Adhesive cells :

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:

- 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).

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).

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2) Transduction Protocol for Suspension Cells:

Grow cells in complete suspension culture medium; use a shaking flask in a CO^2 incubator if necessary.

Measure cell density. When density has reached ~3 x 10^6 cells/ml, measured viability should be > 90%. Dilute cells into 1 x 10^6 cell/ml in complete medium.

Day 1:

- Thaw lentiviral particles at room temperature.
- Add premade lentiviral particles into the diluted cells at a ratio of: 50 to 100 µl virus per 0.5 ml of cells (Note: depending on cell type, you may need to use more or less virus).
- Grow cells in a shaking flask in a CO2 incubator.

Day 2:

At 24 hours after transduction, add an equal amount of fresh medium containing relevant antibiotics. **Note:** amount of antibiotic depends on cell type. Continue growing cells in CO2 incubator.

Day 3:

At 72 hours after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

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.

Note: Filter wavelength settings:

GFP filter: Ex450-490 / Em510-525; RFP filter: Ex545 / Em620;
CFP filter: Ex436 / Em480; YFP filter: Ex500 / Em535;

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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. Wear glove all the time when handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

References:

1. Lee RC, Feinbaum RL, Ambros V (December 1993). "The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14". Cell 75 (5): 843–54;
2. Lewis BP, Burge CB, Bartel DP (2005). "Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets". Cell 120 (1): 15–20;
3. Hammond, S. M. Dicing and slicing: The core machinery of the RNA interference pathway. FEBS Lett. 2005, 579, 5822–5829.;
4. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors. (Link).

Related Products:

GenTarget's Pre-made lentivirus Products:

Product Category	Product Description (please click category name to see product's pages)
Human and mouse ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP**.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla** with different antibiotic selection markers.
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.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing with **CRISPR**
TetR inducible expression repressor	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and

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	antibitoic markers
T-antigen Expression	Express different large and small T antigen with different selection markers
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent marker **GFP/RFP/CFP was sub-cellular localized** in different cell organelle for living cell imaging.
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.
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
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 expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.