GenTarget Inc

6640 Lusk Blvd. Suite A107
San Diego, CA 92121
Phone: (858) 6788683
Fax: (800) 3804198
Orders@gentarget.com

# 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 specific microRNA lentivirus;<br>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:

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.

The **microRNA** (**miRNA**) is a small non-coding RNA molecule found in plants and animals. They are transcripted from RNA precursors (**pre-miRNA**), and matured at around 19 ~ 26 nucleotides in length. There are more than 2000 mature miRNA discovered in human and more than 1200 miRNA in mouse so far. miRNA mainly silence or repression gene expression via binding to the complementary sequences within gene coding mRNA. Each miRNA can target multiple genes. It is believed than >60% mammalian gene

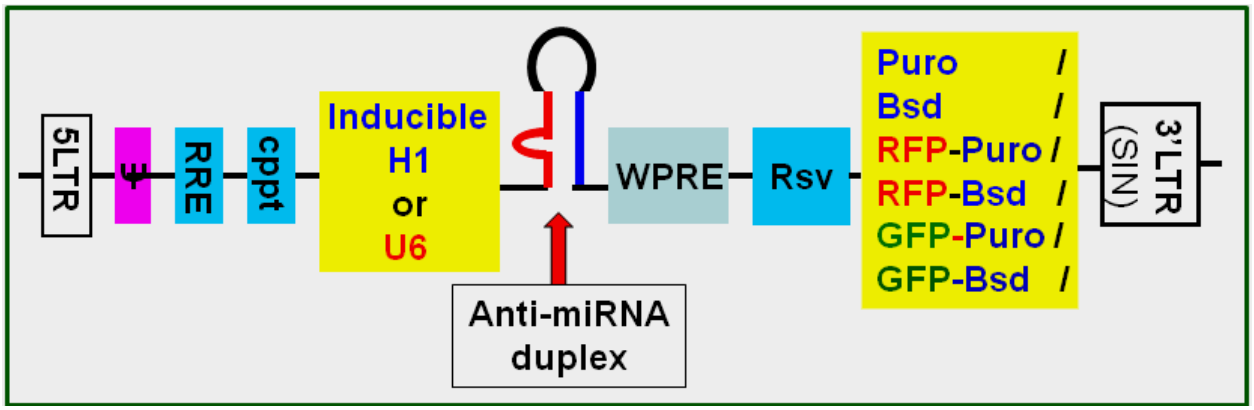
expression was regulated by miRNAs, which involves in most biological processes, including a variety of diseases or disorders development.

**Anti-miRNAi, or miRNA inhibitor** are short RNA molecules complementary to the mature miRNA sequence. Anti miRNA specifically binds to endogenous miRNA which inhibitor or down-regulate miRNA activity. Therefore, anti-miRNA is great tool for the lost of function research of any specific miRNAs. It has potential as therapeutic agents for some biological malfunctions.

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

GenTarget's ready-to-use, anti-miRNA lentiviurs are produced from its optimally designed anti-miRNA lentivectors. The annealed DNA oligonucleotides hairpin duplex encoding the antisense of a specific miRNA sequence, was cloned under a **constitutive** human U6 promoter or under an **optional inducible** human H1 promoter. GenTarget's anti-miRNA lentivector was carefully designed for highest anti-miRNA expression level with precise promoter transcription position, and favor the antisense strand for RISC complex processing. The designed asymmetric duplex transcribes a perfect matched antisense RNA molecular (to miRNA) and leftover a buldged sense RNA molecular. The antisense RNA molecular is expressed in high levels and complementarily "binds" to the endogenous, matured miRNA, which inactivates targeted miRNAs. Please see map scheme below for the anti-miRNA lentivectr's core structure.

**anti-miRNA lentivector core structure:**



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

Gentarget's anti-miRNA lentivirus are featured with **different promoter** (H1 or U6) and **different selection marker** (an antibiotic marker or a fluorescent-antibiotic fusion dual marker), providing tools for long-term constitutively or inducibly inhibit targeted miRNA functionality in long-term stable expression manner. The human H1 and U6 promoter are active in almost all types of mammalian cells. And lentivirus can transduce into majority of mammalian cell types. Thus, anti-miRNA lentivirus is the easiest, most reliable, and consistent delivery method for anti-miRNA molecular.

Gentarget's provides the packaged anti-miRNA lentivirus to any specific human, mouse or any other species' miRNA. Click to see GenTarget's **anti human miRNA list** and **anti mouse miRNA list**.

Each anti-miRNA encodes a specific antisense RNA transcript. A negative control anti-miRNA (**a-miRNA-Neg-control**) lentivirus encodes a deisgned control sequence that has minimal non-specific effects which serves as the negative controls for lentivirus treatment. All lentiviruses demonstrate the strong transduction efficiency. Each virus was validated in lot by lot basis with virus titer at around $1x10^{7-8}$ IFU/ml, and its quality is guaranteed.

The pre-made lentivirus are provided in DMEM medium with 10% FBS and 60ug/ml polybrene as **0.5ml/rach** aliquots in ready-to-use status , or upon request, in PBS solution as ***in vivo ready*** status.

For general questions about our ready-to-use lentiviral particles, please see **FAQ for pre-made lentiviral particles** (.pdf) on our website. (**http://www.gentarget.com/pdf/FAQ-Premade-Lentiviral-particles.pdf**).

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

**3. Why use GenTarget's anti-miRNA lentivirus:**

1. Best delivery method and stable anti-miRNA expression: lentivirus can be effectively transduced into most dividing and non-dividing cells, and the anti-miRNA can integrate into the host cell's genome for stable, long-term expression;
2. The human H1 and human U6 promoter are active in almost all types of mammalian cells. The lentivirus can be used as constitutive high shRNA

expression, or optionally, used as tetracycline inducible shRNA expression (for H1 promoter only);

3. Full coverage: you can order any anti-miRNAs that listed in miRBase from any species, simply provide us the miRNA ID or the mature sequence, or your own validated anti-miRNA sequences;
4. Flexible options with the antibiotic markers, selected from Puromycin, blasticidin, or dual markers as: GFP-puromycin, GFP-blasticidin, RFP-puromycin, RFP-blasticidin.

## Transduction Protocols:

### 1. Adhesive cells Transduction Protocols:

**Note:** the pre-made lentivirus is provided as ready to use. Simply add certain amount of virus into your cell culture. The amount of virus to add is depends on the cell types. A quick transduction protocol is: add 50ul virus into one well in 24-well-plate where cell density is at 50% ~ 75%. At 72 hours after virus added (no need to change medium), visualize the positive rate under fluorescent microscope. For stable cell line generation, pass cell into antibiotic containing medium, or sort the cells via fluorescent signal. Then, select the cells by antibiotics.

**Day 0**: Seed the desired cells in complete medium at appropriate density incubate overnight. (Note: at the time of transduction, it grows to 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. Add fresh, warmed, complete medium (0.5ml). Thaw the Pre-made lentiviral stock at room temperature. Add appropriate amount of virus stock to obtain the desired MOI. Return cells to 37°C/$CO^2$ incubator. (Try to avoid thaw and freeze cycles for pre-made lentivirus. But if you cannot use all virus in one time, you still can re-freeze the virus at -80oC for future use. But virus titer will decrease by ~10% for each re-thaw.)
**Day 3**: At ~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 Guava machine).
**Day 3 +** (optional): Transduced cells can be sorted out via FACS, selected by its specific antibiotics. A pilot experiment should be done to determine

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the antibiotic's kill curve for your specific cell line. (Refer to any literatures about How to generate stable cell lines.)

**2. Suspension cells transduction Protocols:**

1. Grow your cell in your completed suspension culture medium, shaking in flask in $CO^2$ incubator if necessary;
2. Measure cell density. When cell grow to ~3 x $10^6$ cell/ml, measure cell viability (should be > 90%), then diluted cells into 1 x $10^6$ 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: **50 to 100ul virus per 0.5 ml of cells** (Note: depending on the cell types; you may need to use more or less viruses). Grow cells in flask, shaking in CO2 incubator.
4. At 24 hours after transduction, add equal amount of fresh medium containing related antibiotics (Note: each particles contain an antibiotic marker and the antibiotic amounts to use depends upon cell types). Grow cell in $CO^2$ 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).
6. You can sort the fluorescent positive cells, and maintain the antibiotic selection to generate stable cell lines.

**Note: Filter wavelength settings:**

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

## 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).

## 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:

| Product Category | Product Description (please click links below to see product pages) |
|---|---|
| Fluorescent protein | Premade Lentivirus for GFP/ CFP/ YFP/ RFP |
| 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. |
| 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 antibitoic markers |
| Human and mouse ORFs | Premade lentivirus expressin **hundred of human and mouse ORFs** with RFP-Blastididin fusion dual markers. |
| Living cell imging | Pre-made lentivirus particles for Cell Organelle imaging for **Nucleus, Cytoplasm, Endoplasmic Reticulum, Golgi, Mitochondria, Nuclear membrane, Peroxisome, Plasma membrane, Microtubule, Chromatin, Annexin, Actin, Connexin**, and more. |
| Fluorescent-ORF fusion | Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target. |
| shRNA lentivirus | Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more). |
| microRNA | Premade lentivirus expression human or mouse **precursor** |

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| **and anti-microRNA lentivirus** | **miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA. |
| **Negative controls** | 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. |