Pre-miRNA expression lentivirus
User Manual

<table>
<thead>
<tr>
<th>Catalog#</th>
<th>Product Name</th>
<th>Amounts</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNA (a specific micro RNA)</td>
<td>A premade lentivirus expressing a specific human or mouse microRNA with the co-transcribed GFP fluorescent marker and a puromycin antibiotic maker.</td>
<td>1. 0.5 ml of a specific microRNA expression lentivirus;</td>
</tr>
<tr>
<td>H-GFP-miR</td>
<td>Custom-Human miRNA expression lentivirus</td>
<td>2. 0.5ml of Negative control (empty) microRNA lentivirus</td>
</tr>
<tr>
<td>M-GFP-miR</td>
<td>Custom-Mouse miRNA expression lentivirus</td>
<td></td>
</tr>
</tbody>
</table>

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 agent.

The microRNA (miRNA) is a small non-coding RNA molecule found in plants and animals. They are transcribed 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. And more miRNAs are continually discovered. miRNA sequences from different species are summarized in miRBase. miRNA mainly silence or repress gene expression via binding to the complementary sequences within coding mRNA. Each miRNA can target multiple genes. It is believed more than 60% mammalian gene expression is regulated by miRNAs, which involves most biological processes, including a variety of diseases and disorder development.

How is miRNA produced: the miRNA are usually transcribed from RNA poly II promoter (such as CMV or EF1a) whose transcripts are 5' end capped with a poly(A) tail. The endogenous miRNA is initially transcribed as the stem-loop structure in some transcripts which are named as pri-miRNA. The pri-miRNA is enzymatically processed (cleaved), producing an imperfect stem-loop structure, approximately
70-nucleotides in length (named **pre-miRNA**). The pre-miRNA are exported from the nucleus and cleaved in cytoplasm by RNase II enzyme Dicer, yielding an imperfect miRNA:miRNA* duplex about 22 nucleotides in length. The one strand (called **matured miRNA**) is incorporated into the RNA-induced silencing complex (RISC) where it acts as a functional miRNA to interact with the target mRNAs.

2. **pre-miRNA expression lentivirus:**

AMSBIO’s ready-to-use, miRNA expression lentivirus are produced from optimally designed pre-miRNA lentivectors. Human or mouse microRNA precursors and its native context sequences (upstream and downstream flanking genomic sequences) were PCR amplified, and cloned into a **pLenti-TetCMV(GFP-stop-3UTR/miRNA)-Rsv(Puro)** lentivector. The miRNA structure / insert was cloned at the 3'UTR region of the GFP marker. See the scheme below for the core lentivector structure.

The GFP and pre-miRNA are co-transcribed under the same promoter, the **optional inducible CMV promoter**. The **GFP** provides a convenient indicator for miRNA expression levels. A **puromycin** antibiotic selection marker provides the selection method for long term stable expression.

Each miRNA expression insert consists of the native pre-miRNA stem-loop structure and ~300bp downstream and upstream flanking genomic sequence, which ensure the matured miRNA is identical to the endogenous miRNA. AMSBIO provides the pre-packaged specific human or mouse miRNA expression lentiviruses.

Each miRNA encodes a specific pre-microRNA which will be processed *in vivo* into a specific mature microRNA. A negative control (empty) miRNA expression lentivirus (**miRNA-Neg-control**) is also provided to serve as negative control for lentivirus treatment. All lentiviruses demonstrate the strong transduction efficiency. Each virus is validated on 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/each** aliquots in ready-to-use status , or upon request, in PBS solution as **in vivo ready** status.
3. Why use AMSBIO miRNA lentivirus?
   1. Pre-made lentivirus is provided in ready-to-use status and lentivirus are very easy to use by simply adding certain amount of virus into your cell culture. You get the specific miRNA expressed in 24-72 hours. **You do not need to do any miRNA cloning work**, no plasmid prep, no virus production work, no need for lipid transfection.
   2. The best delivery method and stable miRNA expression: lentivirus can be effectively transduced into most dividing and non-dividing cells, and the miRNA expression cassette can integrate into the host cell's genome for stable, long-term expression;
   3. **Constitutive or inducible miRNA expression**: the miRNA lentivirus can be used as constitutive high expression (under the CMV promoter), or optionally, used as tetracycline inducible expression (when teamed with TetR repressor);
   4. **Convenient GFP indicator** for monitoring lentivirus performance and miRNA expression.
   5. **Full coverage**: you can order any human and mouse miRNA expression lentivirus that is listed in miRBase, simply provide the miRNA ID or the mature sequence for the order;

**FAQs:**
1. **Are the pre-miRNA insert fully sequenced?**
   **Answer:** Yes. All pre-miRNA inserts [([~300bp upstream])-(stem-loop-miRNA)-(downstream 300bp)] are fully verified by sequencing analysis. We guarantee the matured miRNA matches to the reference sequence in miRBase. Please be advised the up- and down- stream flanking sequence may contain a few base pair of sequence polymorphisms from the genomic template. This should not affect the sequence of the mature miRNA.

2. **How can I obtain the specific miRNA expression lentivector?**
   **Answer:** Unfortunately we do not sell the generated miRNA expression lentivector plasmid DNA. AMSBIO only provides the pre-made miRNA expression lentivirus. On some special cases, we may provide the specific miRNA expression lentivectors (for customer to produce the miRNA lentivirus by themselves) upon special request.

3. **How to verify the miRNA expression?**
   **Answer:** The miRNA lentivirus contains a GFP maker which is co-transcribed with the miRNA. The GFP fluorescent signal can be conveniently monitored under a fluorescent microscope or any other fluorescent based reader. Therefore, the GFP is the indicator to verify the lentivirus's performance (as well as the miRNA expression) after the virus was transduced. However, to test or verify the matured miRNA expression levels and its specific sequence, you need to perform the RT-PCR and qPCR using specific miRNA primers.
4. Can I generate the miRNA expression stable cell line?
   **Answer:** Yes. It is much easier to generate stable cell line by using lentivirus than by using lipid based transfection. AMSBIO's miRNA expression lentivirus contains a puromycin antibiotic marker for stable cell selection.

5. What is optional inducible expression?
   **Answer:** The miRNA is expressed under an optional inducible CMV promoter. This modified CMV promoter can be used as normal constitutive expression without need for any induction. However, when inducible expression is desired, this promoter can be used as tetracycline inducible expression by introducing the repressor component (tetR) in advance. The TetR initially binds to the modified CMV promoter and stop the expression. Then once the tetracycline is added, the TetR is released from the promoter, which releases the expression. AMSBIO provides the [ready-to-use TetR expression lentivirus](#) (click to see) as standalone catalog products to satisfy the inducible expression needs.

6. What is the negative control miRNA expression?
   **Answer:** We provide the universal negative control miRNA lentivirus for all miRNA expression products. The control virus is produced from the empty miRNA expression lentivector which expresses the same GFP and Puromycin marker from the same lentivector backbone, but does not contain the miRNA structure at GFP's 3'UTR region. The control virus serves as the negative controls for lentivirus treatment in your tests.

**Transduction Protocols:**

1. **Adhesive cells Transduction Protocol:**
   
   **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 dependent on the cell type. 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 is 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 antibiotic.

   **Day 0:** Seed the desired cells in complete medium at appropriate density and incubate overnight. (Note: at the time of transduction, cells should be 50%-75% confluent.)
   For example, seed HeLa cells at $0.5 \times 10^5$/ml x 0.5ml in a well of a 24-well plate;
   **Day 1:** Remove the culture medium. Add fresh, warm, 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 -80°C 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 imaging 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 via FACS and selected by its specific antibiotic. A pilot experiment should be done to determine the antibiotic’s kill curve for your specific cell line. (Refer to any literatures on How to generate stable cell lines.)

2. Suspension cells transduction protocol:
   1. Grow your cells in the complete suspension culture medium, shaking the flask in CO$_2$ incubator if necessary;
   2. Measure cell density. When cell grow to $\sim 3 \times 10^6$ cell/ml, measure cell viability (should be $> 90\%$), then dilute cells to $1 \times 10^6$ cell/ml in complete 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 type, you may need to use more or less virus). Grow cells in flask, shaking in CO$_2$ incubator.
   4. At 24 hours after transduction, add equal amount of fresh medium containing related antibiotic (Note: each particle contains an antibiotic marker and the antibiotic amounts to be used depend upon cell type). 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: $\sim$Ex450-490 $\sim$Em510-525;

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. Wear gloves at all times when handling lentiviral particles! Please refer CDC and NIH’s guidelines for more details regarding to safety issues.

References:
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. 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.

**Related Products:**

<table>
<thead>
<tr>
<th>Product Category</th>
<th>Product Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fluorescent protein</strong></td>
<td>Premade Lentivirus for GFP/ CFP/ YFP/ RFP</td>
</tr>
<tr>
<td><strong>Luciferase expression</strong></td>
<td>Premade lentivirus for all kinds of luciferase protein expression: firefly and Renilla with different antibiotic selection markers.</td>
</tr>
<tr>
<td><strong>CRE recombinase</strong></td>
<td>Premade lentivirus for expressing nuclear permeant CRE recombinase with different fluorescent and antibiotic markers.</td>
</tr>
<tr>
<td><strong>LoxP ColorSwitch</strong></td>
<td>Premade lentivirus expressing ”LoxP-GFP-Stop-LoxP-RFP” cassette, used to monitor the CRE recombination event in vivo.</td>
</tr>
<tr>
<td><strong>TetR inducible expression repressor</strong></td>
<td>Premade lentivirus expresssion TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.</td>
</tr>
<tr>
<td><strong>iPS factors</strong></td>
<td>Premade lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FLF4) factors with different fluorescent and antibiotic markers.</td>
</tr>
<tr>
<td><strong>Human and mouse ORFs</strong></td>
<td>Premade lentivirus expresssion [human and mouse ORFs with RFP-Blastididin fusion dual markers](please click links below to see product pages)</td>
</tr>
<tr>
<td><strong>Living cell imaging</strong></td>
<td>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.</td>
</tr>
<tr>
<td><strong>Fluorescent-ORF fusion</strong></td>
<td>Pre-made lentivirus expression a &quot;GFP/RFP/CFP-ORF&quot; fusion target.</td>
</tr>
<tr>
<td><strong>shRNA lentivirus</strong></td>
<td>Premade shRNA lentivirus for knockdown a specific genes (P53, LacZ, Luciferase and more).</td>
</tr>
<tr>
<td><strong>microRNA and anti-microRNA</strong></td>
<td>Premade lentivirus expression human or mouse precursor miRNA. And anti-miRNA lentivirus and virus for human and mouse miRNA.</td>
</tr>
<tr>
<td>lentivirus</td>
<td>Negative controls</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------</td>
</tr>
<tr>
<td><strong>Premade negative control lentivirus with different markers:</strong> serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression effects.</td>
<td></td>
</tr>
</tbody>
</table>