GenTarget Inc

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Pre-miRNA Expression Lentivirus

Catalog#	Product Name	Amounts
miRNA# (a specific micro RNA #)	A premade lentivirus expressing a specific human or mouse microRNA and a negative miR-control lentivirus	1. 200ul of a specific microRNA expression lentivirus; 2. 200ul of Negative control (GFP only) microRNA lentivirus

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

Product Description:

1. Introduction:

MicroRNA (**miRNA**) are small non-coding RNA molecules found in plants and animals. They are transcribed from RNA precursors (**pre-miRNA**) and mature at approximately 19 - 26 nucleotides in length. More than 2000 mature miRNAs have been discovered in human and more than 1200 in mouse. MiRNAs silence or repress gene expression by binding to complementary sequences within gene-coding mRNA. Each miRNA can target multiple genes. It is believed that >60% of mammalian gene expression is regulated by miRNAs, which involves in most biological processes, including a variety of diseases or disorders development.
The ***miRBase*** database collects and makes available miRNA sequences from a wide range of species.

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

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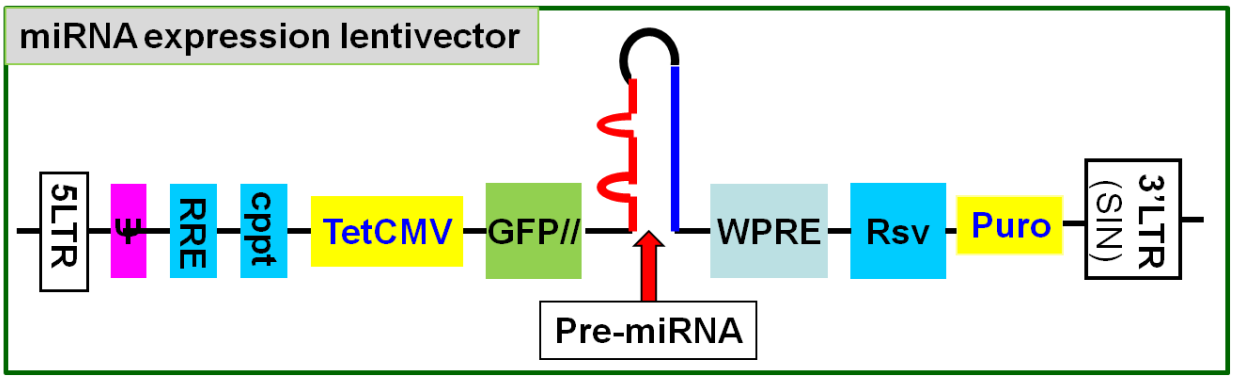
2. **Pre-miRNA expression lentivirus**:

GenTarget's ready-to-use, miRNA expression lentiviruses are produced from our optimally designed pre-miRNA lentivectors. Human or mouse microRNA precursors and native context sequences (upstream and downstream flanking genomic sequences) have been PCR amplified and cloned into a pLenti-TetCMV(GFP-stop-3UTR/miRNA)-RSV(Puro) lentivector and the miRNA structure / insert has been 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*. **GFP** provides a convenient indicator for miRNA expression levels, while the **puromycin** antibiotic resistance marker allows selection for long term stable expression.

Each of GenTarget's miRNA expression inserts consists of the native pre-miRNA stem-loop structure and ~300bp of downstream and upstream flanking genomic sequence, to ensure the mature miRNA is identical to the endogenous miRNA.
GenTarget provides pre-packaged specific human or mouse miRNA expression lentiviruses. Click to see GenTarget's *human miRNA list* and *mouse miRNA list*.

Each miRNA encodes a specific pre-microRNA which will be processed *in vivo* into a specific mature microRNA. All lentiviruses demonstrate strong transduction efficiency. Each lot of virus is validated to have a titer of ~$1x10^7$ IFU/ml, and its quality is guaranteed.

Pre-made lentiviruses are provided in DMEM medium with 10% FBS (premixed with 60µg/ml polybrene) in ready-to-use status.
A negative control (empty) miRNA expression lentivirus (**miRNA-Neg-control**) is also provided.

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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 *anti-miRNA expression lentivirus* for specific miRNA expression; please contact us for a service quote.

Why use GenTarget's miRNA lentivirus?

Ready and Easy to Use: Simply add the virus into your cell culture, and wait for miRNA to be expressed in 24-72 hours. **You do not need to do any miRNA cloning**, plasmid prep, virus production, or lipid transfection, and the lentiviruses can be effectively transduced into most dividing and non-dividing cells.

The most stable method for miRNA expression: The miRNA expression cassette integrates into the host cell's genome for stable, long-term expression.

Constitutive or inducible miRNA expression: The miRNA lentivirus can be used for constitutive high expression under the CMV promoter, or optionally, for tetracycline-inducible expression, when teamed with the TetR repressor.

Convenient GFP indicator for monitoring lentivirus performance and miRNA expression.

Full coverage: You can order an expression lentivirus for any human or mouse miRNA listed in miRBase. Simply provide us with the miRNA ID or the mature sequence.

FAQs:

1. **Is the pre-miRNA insert fully sequenced?**

   Yes. All the pre-miRNA inserts [(~300bp upstream)-(stem-loop-miRNA)-(downstream 300bp)] are fully verified by sequence analysis. We guarantee the mature miRNA to match the reference sequence in miRBase. **Please note:** the up- and down- stream flanking sequence may contain a few base pairs of sequence polymorphism compared to the genomic template. This should not affect the sequence of the mature miRNA.

2. **How can I obtain the specific miRNA expression lentivector?**

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Unfortunately, you cannot. GenTarget does not normally sell the generated miRNA expression lentivector plasmid DNA; we only provide the pre-made miRNA expression lentivirus. In infrequent cases, GenTarget may provide the specific miRNA expression lentivectors on special request.

3. **How do I verify that the miRNA has been expressed?**

   The miRNA lentivirus contains a GFP maker which is co-transcribed with the miRNA. The GFP fluorescence signal can be conveniently monitored using a fluorescence microscope or other fluorescence reader. GFP allows verification of the performance of the lentivirus and miRNA expression. However, to verify and quantify the mature miRNA expression level and sequence specificity, you will need to perform RT-PCR and qPCR using specific miRNA primers.

4. **Can I generate an miRNA expression stable cell line?**

   Yes. It is much easier to generate a stable cell line using lentivirus than by lipid- based transfection. GenTarget's miRNA expression lentivirus contains a puromycin antibiotic marker for stable cell selection.

5. **What is optional inducible expression?**
   The miRNA is expressed under an optional inducible CMV promoter. This modified CMV promoter can be used for normal constitutive expression without the need for induction. However, when inducible expression is desired, this promoter can be used for tetracycline-inducible expression by introducing the repressor protein (TetR) in advance. TetR binds to the modified CMV promoter and stops transcription. Once tetracycline has been added, the TetR is released from the promoter, allowing transcription to proceed. GenTarget provides ready-to-use TetR expression lentivirus separately.

6. **What is the negative control for miRNA expression?**

   GenTarget provides a universal miRNA lentiviral negative control for all of our miRNA expression products. This control virus was produced from an empty miRNA expression lentivector expressing the GFP and puromycin markers on the same lentivector backbone; it does not contain the miRNA structure at the GFP's 3' UTR region.

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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).

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.

Note: Filter wavelength settings:

GFP filter: Ex450-490nm, Em510-525nm;

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.

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 Biosafety 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).

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 humanzied 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).

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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	Llentivirus that express all kinds of infectious antigens with C-term 6His-tag.
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 verifeid 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-miNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Human and mouse ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin 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 fusioned 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

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Unstable GFP	Lentivirus express the 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 Lentiviurs provides the whole-body images with better contrast and brighter images
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 flurescent and antibiotic markers.
CRE, Flp ColorSwtich	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, FLF4**) factors with different fluorescent and antibitoic 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 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.
Ultra titer lentivirus	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.

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