



Product Name: Premade miR#306 lentivirus

Product Name: miRNA expression lentivirus for human microRNA:
hsa-mir-375 (MI0000783).

Catalog number: miR#306

Summary:

Human hsa-mir-375 (MI0000783) microRNA expression lentivirus (0.5 ml) and the negative control miRNA lentivirus (0.5 ml).

Packed items:

miRNA expression lentivirus (total 2 tubes)	
Tube label	Amount delivered
miRNA expression lentivirus: hsa-mir-375 (GFP / Puro) 0.5 ml x (1x10 ⁷ IFU/ml)	0.5 ml/each x 1 tube
miRNA Control lentivirus: miRNA-Control (GFP / Puro) 0.5 ml x (1x10 ⁷ IFU/ml)	0.5 ml/each x 1 tube

Storage: Store premade lentivirus in -80°C. Lentivirus is stable for > 6 months when stored appropriately.

Product details:

1. miRNA expression lentivector insert sequence:

Human **hsa-mir-375** microRNA precursor sequence and its native genomic context sequences (100bp of upstream and downstream genomic flanking sequences) was synthesized. The synthesized miR expression insert sequence is showed below (note: the red highlighted region is the **hsa-mir-375** miR precursor sequence):

```

GGCGGAGGCTAGCGGGGCGCTGTGCAGCACTGAGCTCGCGGAAGACCAGGA
CCAGGAGATACCGAGGGCGACCGCCAGGCCCGGGCCCTCCGCTCCCGCCC
CGCGACGAGCCCCTCGACAAACCGGACCTGAGCGTTTTGTTTCGTTTCGGCTCG
CGTGAGGCAGGGGCGGCCTCTCAGCACCAGCCCGGGGGCCGGCCTGATCGCC
ACGCAGGCACCTGCCGCCGCCACCGCCACCGCCATCTCAACCGTACGGGTGG
GAGA

```



2. miRNA precursor sequence:

hsa-mir-375, [MI0000783](#) miRNA stem-loop sequence is listed below:

```
CCCCGCGACGAGCCCCUCGCACAAACCGGACCUGAGCGUUUUUGUUCGUUCGGCUCGCGUGAGGC
```

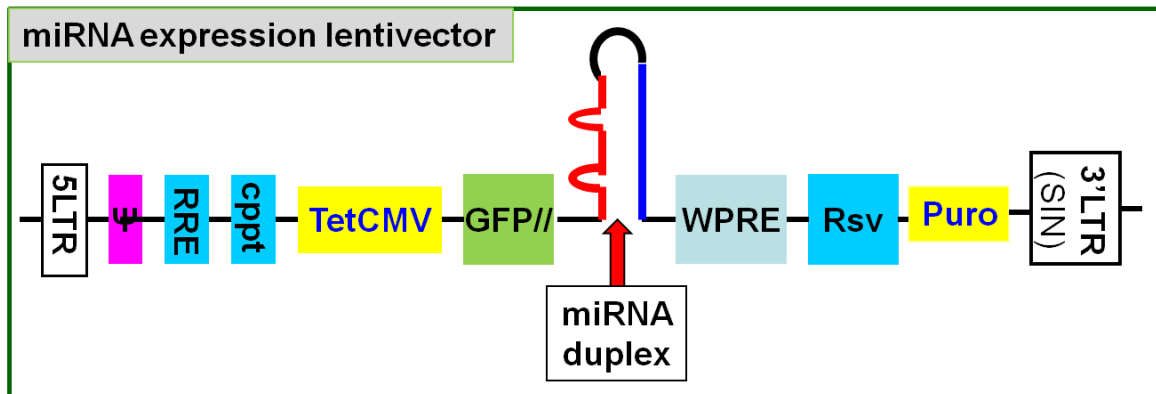
It guarantees generating a matured microRNA, **hsa-mir-375**, with 22 bp in length, listed below:

```
UUUGUUCGUUCGGCUCGCGUGA
```

3. miRNA expression lentivector:

The synthesized double stranded miRNA expression insert (embedded **hsa-mir-375** microRNA) was cloned into GenTarget's **miRNA expression lentivector**. The miR-control lentivector that contain a Null sequence was also made in the same vector backbone, which serves as the miRNA-control.

The miRNA expression insert was cloned at 3' UTR region of GFP marker, transcribed under an **optional inducible** CMV promoter (TetCMV). The miRNA lentivector also contains a puromycin antibiotic marker under a RSV promoter. Please see the vector scheme below for the miRNA expression lentivector's core structure.



For more details about GenTarget's miRNA, please refer to website (www.gentarget.com under [miRNA lentivirus](#)).

4. miRNA expression lentivirus production:

miRNA expression lentivector was co-transfected with lentiviral packaging plasmids (Cat#: **HT-pack**) into lentivirus production cell line (Cat#: **TLV-C**). Lentiviruses were packaged in DMEM medium with 10% serum following



GenTarget's production protocols. Viruses will be filtered through 0.45 um filter, and delivered as 0.5ml aliquots.

5. Lentivirus titer measurement:

Virus titers were measured via GFP fluorescent cell counting post transduction into HT1080 cells. (Note: Titers may vary dependent upon cell types, assay methods, and the RSV promoter's strength in your cell type which drives the GFP marker).

Titer results:

Virus Name	Titer (IFU/ml)
miRNA expression lentivirus: hsa-mir-375	1.01 x 10 ⁷
miRNA control lentivirus: miRNA-Control	1.38 x 10 ⁷

About the premade lentiviral particles:

Delivered expression lentiviral particles are in ready-to-use status. Simply add certain amount of particles into your cell of interest. The amount of particles added is dependent the cell types. The expression will be peaked at 72 hours post transduction in most cell line. For general transduction reference, please review the protocol at our website:

<http://www.gentarget.com/pdf/Transduction-protocols.pdf>

For FAQ about premade lentiviral particles, please refer to our website at:

<http://www.gentarget.com/pdf/FAQ-Premade-Lentiviral-particles.pdf>

About Optional Inducible Expression:

Please be advised that the premade lentivirus can be used for constitutive high expression of miRNA without needs for induction. However, the miR was driven by an tetracycline inducible human CMV promoter. Optionally, it can be used as tetracycline inducible expression. For inducible expression, a Tetracycline repressor protein must be present in advanced to inhibitor the expression. The repress protein can be delivered by co-transfected with a repressor expression plasmid, or by co-transduction with the Tet- repressor expression lentiviral particles. Gentarget provides the [Tet-repressor lentiviral particles](#) with different antibiotic markers. Note: For inducible expression, GenTarget also provides [TetR repressor stable cell lines](#) with different antibiotic marker.



When used as tetracycline inducible expression (i.e. the repressor was present in advance), the expression is induced after Tetracycline is added into the culture. The amount of tetracycline to use is dependent upon cell types, a common used final concentration is 1-2 ug/ml. Be aware of that many bovine serum used in culture are contaminated with tetracycline or its derivatives which can affect basal expression. If desired, use Tetracycline-Free (certified) FBS in cell culture when inducible expression is required. The induction rate is from 10 to 1000 folds dependent upon cell types.

Warranty and terms:

- Delivered lentivirus was guaranteed free of bacterial and mycoplasma contamination;
- The delivered miRNA expression lentivirus was guaranteed with a virus titer at around 1×10^7 IFU/ml, measured in common cell types via GFP positive cell counts. (Note: titer could be different depending on cell types and assay conditions.)
- The delivered products are for research use only, not intend to be used in therapeutics or diagnostics;

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 at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.