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# Product Information

**MISSION® Lenti-miRs – Lentiviral Constructs for miRNA Expression**

Catalog Numbers **HLMIR0001 – HLMIR2781, MLMIR0001 – MLMIR2049**

Storage Temperature -70 °C

**Product Description**

MicroRNAs (miRNAs) are a class of genome-encoded nucleic acids that have been shown to regulate gene expression in a variety of organisms. These small, noncoding RNA molecules function by mediating transcript degradation, inhibiting translation, or a combination of these mechanisms. This type of regulation generally occurs by base pairing of the miRNA to a target sequence within the 3' UTR of a transcript. Thousands of miRNAs have been identified and classified via sequencing or bioinformatics approaches that are based on strongly conserved sequence motifs. The University of Manchester operates the publicly available *miRBase Sequence Database*, where microRNA data are managed and annotated.

Sigma's Mission Lenti-miRs express miRNAs from a common backbone, whose structure meets requirements for accurate Dicer processing.[1] Mature microRNA sequences are obtained from miRBase, and a partially complementary strand is designed to mimic the base pairing pattern in the backbone structure using a proprietary algorithm. Oligos containing the microRNA sequences are cloned into the TRC2-pLKO-puro vector (see figure 1). Each miRNA construct has been cloned and sequence verified.

Lentiviral transduction particles are produced from sequence-verified lentiviral plasmid vectors. Oligos containing the microRNA sequences are cloned into the TRC2-pLKO-puro vector (see figure 1). Co-transfection of this vector into the appropriate cell line with compatible packaging plasmids produces viral particles that can be used to transduce mammalian cells. The polymerase II promoter, elongation factor 1 alpha (EF1A), was chosen to drive miRNA expression.[2] The vector also contains elements needed for reverse transcription of viral RNA and integration of viral DNA into the host cell genome. Additionally, the Woodchuck Hepatitis Post-Transcriptional Regulatory element[3] (WPRE) is included, allowing for enhanced expression of transgenes delivered by lentiviral vectors.[4] This lentiviral vector also carries a puromycin resistance gene for selection of cells. Unlike murine-based MMLV or MSCV retroviral systems, lentiviral-based particles permit efficient infection and integration of the specific miRNA construct into differentiated and non-dividing cells, such as neurons and dendritic cells,[5] overcoming low transfection and integration difficulties when using these cell lines. Self-inactivating replication incompetent viral particles are produced in packaging cells (HEK293T) by co-transfection with compatible packaging plasmids.[6-7]

In addition, the lentiviral transduction particles are pseudotyped with an envelope G glycoprotein from Vesicular Stomatitis Virus (VSV-G), allowing transduction of a wide variety of mammalian cells including primary and embryonic stem cells.[8] The lentiviral transduction particles are titered via a p24 antigen ELISA assay and pg/ml of p24 are then converted to transducing units per ml using a conversion factor.

To use Lenti-miRs, one needs to first select a cell type in which the miRNA of interest is expressed at low or undetectable levels. If unsure about the levels of miRNA expression in a particular cell line, it is critical that you determine those levels by conducting qRT-PCR or similar assay. We recommend using the MystiCq™ microRNA cDNA synthesis Mix. Starting with total RNA or RNA preparations pre-enriched for microRNAs, this kit provides all the components necessary to convert mature miRNAs into cDNA templates for qPCR. Once miRNAs have been chosen and quantified in the cell line of choice, cells can be transduced with lentiviral particles containing the miRNAs.

MISSION Lenti-miR activity may be assessed by the Dual-Luciferase Assay System which requires co-transfecting a Dual-Luciferase® Reporter (DLR™) plasmid with a complementary miRNA binding sequence (see Figure 2 in Appendix). Levels of target mRNA or protein can be assessed using a qRT-PCR assay or western Blotting, respectively.

**Components/Reagents**
The individual constructs are provided in Dulbecco's Modified Eagle's Medium with 10% heat-inactivated fetal bovine serum and penicillin-streptomycin.

Standard volume: 0.2 mL

**Precautions and Disclaimer**
These products are for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices. Though the lentiviral transduction particles produced are replication incompetent, it is highly recommended that they be treated as **Risk Group Level 2 (RGL-2)** organisms.[9] Follow all published RGL-2 guidelines for handling and waste decontamination.

**Storage/Stability**
All components are stable for at least six months after receipt when stored at -70 °C. Avoid repeated freeze/thaw cycles, which will severely reduce functional viral titer.

**Related Products**
- Hexadimethrine Bromide, Catalog Number H9268
- Puromycin dihydrochloride, Ready Made solution, 10 mg/ml in $H_2O$, Catalog Number P9620
- Minimum Essential Medium containing 10% fetal calf serum or growth medium optimized for the specific cell line
- MISSION ExpressMag® 96-Well Magnetic Kit, Catalog Number SHM02
- MystiCq® microRNA cDNA Synthesis Mix, Catalog Number MIRRT
- KiCqStart® SYBR® Green qPCR ReadyMix™, Catalog Number KCQS00
- mirPremier® microRNA Isolation Kit, Catalog Number SNC10, SNC50
- MISSION microRNA Mimics
- MISSION Lenti microRNA Inhibitors
- MISSION Synthetic microRNA Inhibitors
- MISSION TRC2 pLKO.5-puro-CMV-TurboGFP™ Positive Control Transduction Particles, Catalog Number SHC203V
- MISSION pLKO.1-puro Non-Mammalian shRNA Negative Control Transduction Particles, Catalog Number SHC002V
- MISSION Lenti microRNA, ath-miR416, Negative Control 1 Transduction Particles, Catalog Number NCLMIR001
- MISSION Lenti microRNA, cel-miR-243-3p, Negative Control 2 Transduction Particles, Catalog Number NCLMIR002

**Procedure for the Use of MISSION Lentiviral microRNA Transduction Particles:**

**Puromycin titration (kill curve)** should be performed before starting transduction when working with a new cell type.

1. Plate 1.6 x $10^4$ cells into wells of a 96-well plate with 120 µl fresh media.
2. The next day add 0.5-10 µg/ml of puromycin to selected wells.
3. Examine viability every 2 days.
4. Culture for 3 – 14 days depending on the growth rate of the cell type and the length of time that cells would typically be under selection during a normal experimental protocol. Replace the media containing puromycin every 3 days. The minimum concentration of puromycin that causes complete cell death after the desired time should be used for that cell type and experiment.

**Day 1**
Seed the mammalian cell line of choice in complete medium into culture dishes or multiwell plates 24 hours prior to transduction. Plating density should be such that cells are 50-80% confluent at the time of transduction.

**Day 2**
Thaw the lentiviral stock slowly on ice. Gently spin down material in tubes before opening. Add hexadimethrine bromide (the chemical equivalent of Polybrene) to the cells at a final concentration of 8 µg/ml.
**Note**: Hexadimethrine bromide enhances transduction of most cell types. However, some cells, such as primary neurons, are sensitive to hexadimethrine bromide. When using sensitive cells, do not add the hexadimethrine bromide and the cells should still be transduced.

Following addition of hexadimethrine bromide, gently swirl the plate to mix. Add the appropriate amount of viral particles at a suitable multiplicity of infection (MOI) and swirl the plate gently to mix. Incubate the cell-viral particle mixture at 37 °C overnight.

**Multiplicity of Infection** (MOI) is the number of transducing lentiviral particles per cell. It is highly recommended that for each new cell type to be transduced, a range of MOI be tested.
To calculate MOI: (total number of cells per well) x (desired MOI) = total transducing units needed (TU)

(total TU needed) / (TU/ml reported on C of A) = total ml of lentiviral particles to add to each well

**Notes**
a. When transducing a lentiviral construct into a cell line for the first time, it is recommended that a range of MOIs (0.5-20) be used to find the optimum degree of transduction efficiency.
b. When overnight incubation presents a toxicity concern, cells may be incubated for as little as 4 hours before changing the medium. Cells can be transduced in reduced medium volumes to increase transduction efficiencies.

**Day 3**
Remove the viral particle-containing medium and replace it with fresh, pre-warmed complete culture medium.

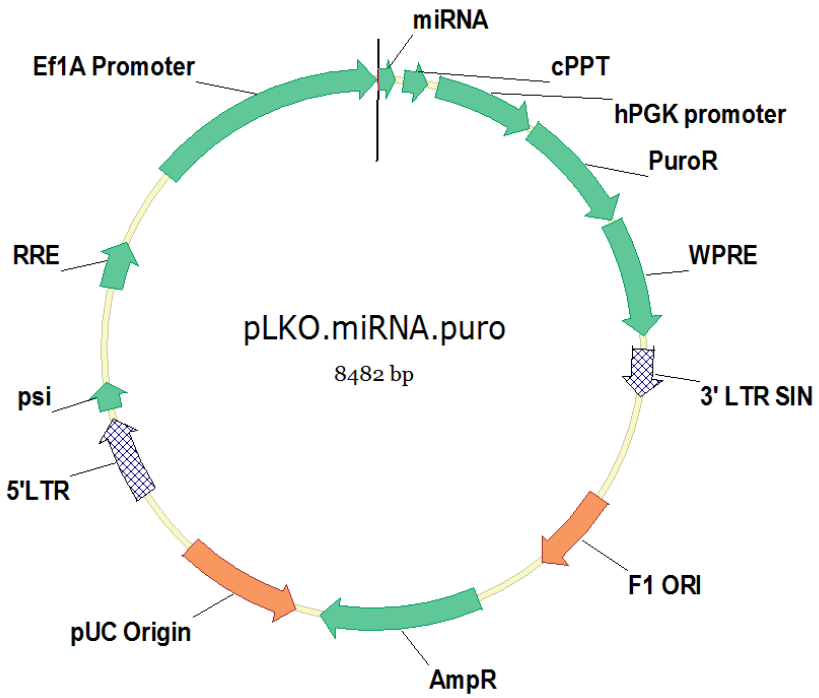
**Day 4**
For puromycin selection, remove the medium and replace it with fresh, complete medium that contains the appropriate amount of puromycin for selection of transduced cells. Proceed to day 5.
**Note:** When the appropriate concentration of puromycin for a specific cell type is unknown, perform a kill curve experiment, as described above. Typically, puromycin concentrations ranging from 0.5-10 µg/ml are sufficient to kill most untransduced mammalian cell lines. Excess puromycin will cause undesired phenotypic responses in most cell types.

**Day 5 and forward**
Replace medium with fresh, puromycin-containing medium every 3-4 days until resistant colonies can be identified (generally, 10-12 days after selection). If isolated colonies are desired, pick a minimum of 5 puromycin-resistant colonies and expand each clone to assay. Alternatively, miRNA expression and function may be evaluated with a puro-selected culture, containing all transduced cells.
**Note:** Due to the random integration of the lentivirus into the genome, varying levels of miRNA may be seen from different puromycin-resistant clones. Testing a number of puromycin-resistant clones will allow a determination of which clone is optimal.

**Assessing miRNA expression and function**
miRNA expression from Lenti-miRs can be evaluated by RT-qPCR. We recommend the MystiCq® microRNA cDNA Synthesis Mix and MystiCq® microRNA qPCR assay primers. To evaluate function, reporter assays or levels of target protein and/or messenger RNA levels may be evaluated. miRNA functional studies may require simultaneous analyses of both mRNA and protein expression. While qRT-PCR can be used to assess levels of target transcript, Western analysis or other validated immunoassays are used to investigate the impact on protein quantity. Reporter assays, such as a dual luciferase reporter assay, are used to study the interaction between miRNAs and their target sites.

**Figure 1. TRC2 Lentiviral Plasmid vector TRC2-pLKO-puro Features**

| Name | Description |
|---|---|
| EF-1alpha | Elongation factor-1alpha Promoter |
| cppt | Central polypurine tract |
| hPGK | Human phosphoglycerate kinase eukaryotic promoter |
| PuroR | Puromycin resistance gene for mammalian selection |
| WPRE | Woodchuck Hepatitis PostTranscriptional Regulatory element |
| SIN/3' LTR | 3' self inactivating long terminal repeat |
| f1 ori | f1 origin of replication |
| AmpR | Ampicillin resistance gene for bacterial selection |
| pUC ori | pUC origin of replication |
| 5' LTR | 5' long terminal repeat |
| Psi | RNA packaging signal |
| RPE | Rev response element |
| miRNA | miRNA sequence (clone specific) |



**Troubleshooting Guide**

| Problem | Cause | Solution |
|---|---|---|
| No Transduction of cells | Viral stock stored incorrectly | Store stocks at -70 °C. Do not freeze/thaw more than 2 times. |
| Low miRNA expression detected due to low transduction efficiency | Population of cells not transduced efficiently | Select for transduced cells with puromycin, and allow selected population to become mitotic. |
| | Hexadimethrine bromide not included during transduction | Transduce in the presence of hexadimethrine bromide. |
| | Non-dividing cell type used | Transduce at a higher MOI, or evaluate transduction enhancement reagents, such as Express Mag. |
| | It is unknown how efficiently cells can be transduced with VSV-G pseudotyped lentiviral particles | Try transducing cells with control lentiviral particles such as MISSION® TRC2 pLKO.5-puro-CMV-TurboGFP™ Positive Control Transduction Particles (SHC203V) to establish experimental parameters for transductions. |
| | Cells were harvested and assayed too soon after transduction | Harvest cells 72 hours after transduction, and not earlier. Alternatively, results may be improved by placing cells under puromycin selection because untransduced cells will be killed. |
| Low miRNA impact due to target choice or cell line variability in expression of the miRNA | miRNA is not expressed at a high enough level for analysis in cell line | Evaluate miRNA levels in cell type of choice via qRT-PCR using the MystiCq™ microRNA cDNA Synthesis Mix kit.<br>Consider alternative cell lines if endogenous miRNA levels are high.<br>Use a combination of assays to test the miRNA target, such as qRT-PCR, Western analysis, and reporter assays such as a dual luciferase assay |
| No signal from reporter assay (such as a dual luciferase assay) | Reporter assay might not be working correctly | Double check to make sure reporter plasmid target sequence and orientation is correct. |
| Cytotoxic effects observed after transduction | Expressed miRNA causes cell cycle arrest or apoptosis | Be sure expressed miRNA does not have an adverse phenotype on your cell line of interest. |

| | | |
|---|---|---|
| Cytotoxic effects observed after transduction (continued) | Hexadimethrine bromide was used during transduction | Be sure that cells are not sensitive to hexadimethrine bromide. Omit the hexadimethrine bromide during the transduction. |
| | Too much puromycin was used for selection | Determine the puromycin sensitivity of the cells by performing a kill curve and use the minimum concentration required to kill the untransduced cells. |

**Control Selection Table**

| **Recommended Control** | **Objective** |
|---|---|
| Negative Control: Untreated Cells | Untreated cells will provide a reference point for comparing all other samples. |
| Negative control: Transduction with lentiviral particles containing a sequence targeting no known mammalian genes (designed against Turbo GFP sequence), Catalog Number SHC002V | MISSION® pLKO.1-puro Non-Mammalian shRNA Control Transduction Particles provides a reference point to compare lentiviral transduced samples. |
| Negative control: Transduction with lentiviral particles containing a sequence targeting no known mammalian genes, Catalog Number SHC016V | MISSION® pLKO.1-puro Non-Mammalian shRNA Control Transduction Particles provides a reference point to compare lentiviral transduced samples. |
| Negative control: Transduction with MISSION Lenti microRNA, ath-miR416, Negative Control 1, Sequence from *Arabidopsis thaliana* with no homology to human and mouse gene sequences, Catalog Number NCLMIR001 | MISSION® pLKO.miRNA-puro Non-Mammalian microRNA Control Transduction Particles provides a reference point to compare lentiviral transduced samples. |
| Negative control: Transduction with MISSION Lenti microRNA, cel-miR-243-3p, Negative Control 2, Sequence from *Caenorhabditis elegans* with no homology to human and mouse gene sequences, Catalog Number NCLMIR2 | MISSION® pLKO.miRNA-puro Non-Mammalian microRNA Control Transduction Particles provides a reference point to compare lentiviral transduced samples. |
| Positive control: Transduction with lentiviral particles containing Turbo GFP, Catalog Number SHC203V | MISSION® TRC2 pLKO.5-puro-CMV-TurboGFP™ Positive Control Transduction Particles can be used to monitor transduction efficiency. |

**Cell Type Table**

The cell types listed below have been successfully infected by pLKO.2-puro based lentiviral particles. Optimal conditions will need to be determined for your experimental needs. For the most updated cell line list, and some guidelines for conditions, please visit:

http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/shrna/learning-center/getting-started.html

| **Cell lines, human** | **Cell Type** | **Cell lines, human** | **Cell Type** | **Primary cells human** | **Cell Type** |
|---|---|---|---|---|---|
| HEK293 | embryonic kidney cells | A431 | epidermal carcinoma | dendritic | immature dendritic |
| HeLa | cervical adenocarcinoma | THP1 | monocytic | T-cells | lymphocytes |
| A549 | lung adenocarcinoma | RAW264.7 | macrophage | epithelial | prostate |
| H1299 | lung carcinoma | SH-SY5Y | brain neuroblastoma | fibroblasts | primary mammary |
| HT29-D4 | colon carcinoma | HCN-1A | brain cortical neuron | **Primary cells, other species** | **Cell Type** |
| HepG2 | hepatocellular carcinoma | SupT1 | T-cells | ECS | mouse embryonic stem cells |
| HCT116 | colon carcinoma | BJ-TERT | diploid fibroblasts | fibroblasts | Mouse embryonic fibroblasts |
| MCF7 | breast carcinoma | **Cell lines, mouse** | **Cell Type** | MC3T3-E1 | mouse bone marrow derived |
| MCF10A | breast carcinoma | NIH3T3 | fibroblast | molar mesenchymal | mouse embryonic mesenchymal |
| Panc-1 | pancreatic epitheliod carcinoma | **Primary cells, human** | **Cell Type** | cardiomyocytes | rat neonatal cardiomyocytes |
| PC3 | prostate carcinoma | astrocytes | normal | | |
| DU145 | prostate carcinoma | C3H10T1/2 | mesenchymal | | |

**References**

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9. NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) 2002 (http:11www4.od.nih.gov/oba).





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## Appendix

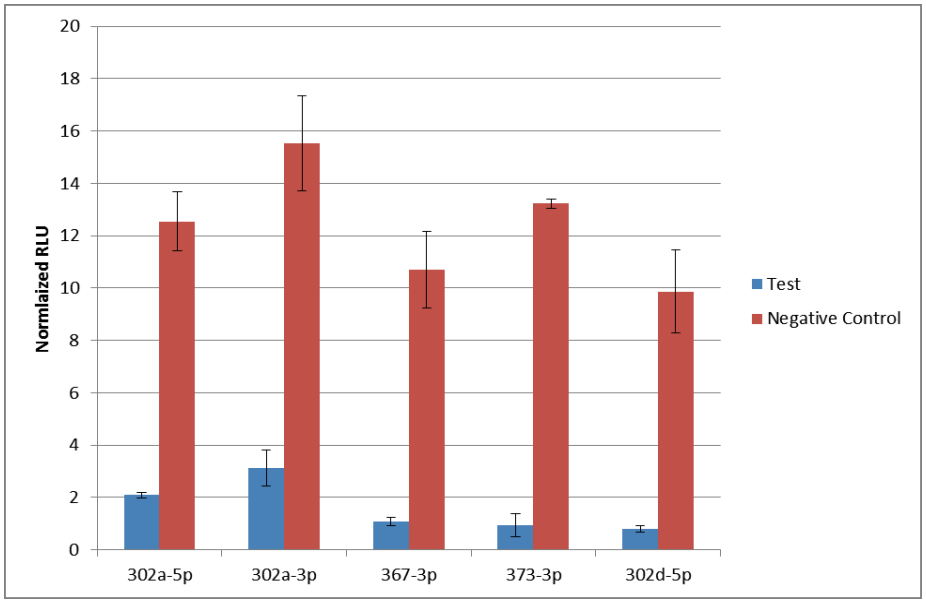


**Figure 2**. Lenti-miRs were transduced into HeLa cells. After two weeks under puromycin selection, the Dual-Luciferase® Reporter (DLR™) plasmid, with a complementary miRNA binding sequence, was transfected into the cells. miRNA activity is shown for 5 miRNAs, as compared to a negative control, using the Dual-Luciferase Assay System.