mirPS® Liposomal Transfection Kit
Product Data Sheet

The miR-302/367 liposomal transfection kits facilitate the efficient delivery of miR-302 clusters into target cells. The kits contain miR-302 vectors and transfection agent that can easily transport the miR-302 clusters into target cells without using additional equipment. The inclusion of GFP and Puromycin resistance enables simple identification and selection of transfected cell colonies.

Cells that have successfully been reprogrammed:
- Hair Follicle Cells
  - Keratinocyte
  - Melanocyte
  - Fibroblast
- Colo (Melanoma)
- HepG2 (Liver Cancer)
- MCF7 (Breast Cancer)
- HEK-293
- Mesenchymal Stem Cell
- PC3 (Prostate Cancer)
- Tera-2 (Embryonal Teratoma)

Product Catalog

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Product Name</th>
<th>Price</th>
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<tbody>
<tr>
<td>M-125-302-CM</td>
<td>mirPS® mir-302 Liposomal/Polysomal Kit (5-run)</td>
<td>$880</td>
</tr>
<tr>
<td>M-126-302-CM</td>
<td>mirPS® mir-302 Liposomal/Polysomal Kit (10-run)</td>
<td>$1,580</td>
</tr>
<tr>
<td>M-127-302-CM</td>
<td>mirPS® mir-302/367 Liposomal/Polysomal Kit (5-run)</td>
<td>$880</td>
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<tr>
<td>M-128-302-CM</td>
<td>mirPS® mir-302/367 Liposomal/Polysomal Kit (10-run)</td>
<td>$1,580</td>
</tr>
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Contents

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<th>Reagents</th>
<th>5-run Volume</th>
<th>10-run Volume</th>
<th>Ingredients</th>
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<tr>
<td>1 miR-302 or miR-302/367 Plasmid</td>
<td>75 µL</td>
<td>150 µL</td>
<td>miR-302 or miR-302/367 vector DNA in Tris buffer (pH7.4)</td>
</tr>
<tr>
<td>2 Control Plasmid</td>
<td>75 µL</td>
<td>150 µL</td>
<td>Blank vector DNA in Tris buffer (pH7.4)</td>
</tr>
<tr>
<td>3 Transfection Reagent</td>
<td>250 µL</td>
<td>500 µL</td>
<td>Liposome/Polysome</td>
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Call (877)700-0RNA or visit [www.mellobiotech.com](http://www.mellobiotech.com) for more information

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Liposomal Kit
For delivery of miR-302 (only) or miR-302/367 into mammalian cells.

Included in this kit:
- miR-302 or miR-302/367 vector
- Control vector
- Transfection reagent

PROTOCOL

Step 1—Cell Preparation:
1. Seed appropriate number of mammalian cells onto a 100-mm tissue culture dish. The number of cells required varies with cell type. For best results, wait until cells have adhered to the plate’s surface and are about 40-60% confluent on the day of transfection.
2. One hour prior to transfection, replace the cell culture medium with 10 ml of fresh serum-free/antibiotic-free medium.

Step 2—Transfection:
1. Into a sterile 1.5 mL microcentrifuge tube, add 15 µL of miR-302 Plasmid (#1) or Control Plasmid (#2).
2. Add 1 mL of serum-free/antibiotic-free medium and mix well by vortexing. Spin tube briefly.
3. Add 50 µL of Transfection Reagent (#3) to the center of the 1.5 mL microtube. Add directly into the liquid in the tube. Do not pipette the transfection reagent onto the sides of the tube!
4. Vortex for at least 30 seconds.
5. Incubate for 10-15 minutes at room temperature. (The transfection mixture can be incubated for longer at room temperature, but do not exceed 30 minutes.)
6. Add the mixture drop-wise, covering the entire surface of the 100-mm culture dish. Mix by shaking the culture dish back-and-forth and right-to-left several times to evenly distribute the transfection mixture.
7. Incubate the cells at 37°C under 5% CO₂ for 12-18 hours and then replace the medium with FBS containing growth medium.
8. Continue to grow the cells at 37°C under 5% CO₂ until the green-positive cells can be observed under a fluorescent microscope. GFP expression is typically observed 1-2 days post-transfection. The results may vary with different cell types.

Step 3—Selection of Positive Cells:
1. Once GFP expression has been observed, puromycin selection may be used to remove any non-transfected cells. Treat cells with 50-100 µg/mL puromycin for 24-48 hours. Observe cells; if a large percentage of non-transfected cells remain, increase the puromycin concentration and treat for 24-48 hours or until population is relatively pure.
2. Once iPSC colonies are larger, select colonies (either manually with a pipette or with a micromanipulator) and transfer them to a new tissue culture dish containing Feeder-Free Culture Medium (Mello Bio cat. no. M-800-011). Allow colonies to grow to desired size.

Troubleshooting

Does the miR-302/367 vector integrate into the genome?
Integration depends on the delivery method. Liposomal transfection is transient in nature, and it should not integrate into the genome. Transfection by electroporation should not cause integration. However, under conditions of high voltage or long duration, some integration (<10%) may occur. Lentiviral infection leads to integration.

How can I improve the transfection efficiency?
GFP should be observed within 1-2 days after transfection. Some cells are difficult to transfect and may require multiple transfections. Low transfection efficiency may be the result of multiple factors including:
- Inappropriate cell density at the time of transfection
- Inadequate incubation time for the transfection complex
- Poor cell condition/old cells
If you are experiencing problems with liposomal transfection, try increasing or decreasing the amount of transfection complex used per reaction. Mello Biotech also offers an electroporation kit, which may work better with some cell lines.

What is the morphology of a miR-302-induced pluripotent stem cell?
The iPSCs are spherical cells with loose nuclei and are surrounded by abundant cytoplasm.

I have initial high transfection efficiency, but very few iPSC colonies. How can I increase the reprogramming efficiency?
Reprogramming is largely dependent on cell type and concentration of miR-302 (Lin et al., NAR 2011). Make sure puromycin selection is performed. Wait 3-4 days after Puromycin selection before transferring colonies to feeder-free medium. If you switch cells to feeder-free medium too soon, cells may detach and form aggregates which may reduce iPSC survival. We recommend culturing iPSC colonies under feeder-containing conditions to improve reprogramming efficiency.

How can I isolate an iPSC colony to produce a cell line?
A two- to eight-cell stage iPSC colony is the best choice to isolate for the formation of a single iPSC line. Cell isolation can be performed under an inverted microscope using a micromanipulator in conjunction with a micro-injector holder. Alternatively, you can select colonies with pipette and very sharp eyes. Fluorescence-activated cell sorting (FACS) can also be used to isolate iPSC colonies.

How do I ensure the quality of miR-302 vector?
Store the kit at -80°C freezer to maintain its quality. The vector is stable for one year under this condition. Avoid repeated freeze-thaw cycles of the vector. Due to the structural complexity of the miR-302 cluster, amplification of the vector may cause mutations. If you would like to sequence the vector to insure its quality, please contact Mello Biotech for primer information.

Refer to the FAQ section of our website (http://www.mellobiotech.com/faq.html) and Lin et al. (2011) Nucleic Acids Research 39: 1054-1065 for additional information on miR-302/367 reprogramming.
Introduction

The miR-302 Liposomal Expression Kit is designed for intracellular delivery and expression of the miR-302 microRNA family (miR-302b, c, a and d pre-microRNA cluster, with or without miR-367). As shown in Figure 1, expression of the miR-302 pre-microRNA is cytomegalovirus (CMV) promoter-driven and directly linked to GFP expression, allowing easy identification of transfected cells. In addition, the vector includes a puromycin resistance gene for selection of target cells.

The miR-302 family is expressed most abundantly in early human embryonic stem (hES) cells and quickly decreases after cells differentiate. Transgenic delivery of miR-302’s into human skin, hair follicle and kidney epithelial cells, as well as prostate cancer, breast cancer, liver cancer, and melanoma cells has been shown to orchestrate the reprogramming of somatic cells to induced pluripotent stem (iPS)-like cells (References 1, 2 and 3). The miR-302-reprogrammed iPS cells, namely miR-PS cells, not only express many key hES cell markers, such as Oct4, Sox2 and Nanog, but also have a highly demethylated genome similar to hES cells. Microarray analyses revealed that genome-wide gene expression patterns between the miR-5 and hES H1 and H9 cells share over 86%–92% similarity. Under molecular guidance in vitro, these miR-PS cells can differentiate into many distinct tissue cell types derived from all three germ layers. Therefore, miR-302 can replace Oct4, Sox2 and Nanog for simpler and more efficient generation of iPS cell lines.