Using Liposome-mediated Transfection for Gene Delivery

Introduction
This protocol describes how to deliver plasmid DNA into iCell® Cardiomyocytes using the ViaFect Transfection Reagent.1,2

Required Consumables
The following consumables are required in addition to the materials specified in the iCell Cardiomyocytes User’s Guide.

<table>
<thead>
<tr>
<th>Item</th>
<th>Vendor</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>iCell Cardiomyocytes Kit</td>
<td>Cellular Dynamics International (CDI)</td>
<td>CMC-100-010-001, CMC-100-010-005</td>
</tr>
<tr>
<td>Opti-MEM Reduced Serum Medium</td>
<td>Life Technologies</td>
<td>31985-062</td>
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<tr>
<td>Plasmid DNA</td>
<td>Multiple Vendors</td>
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<tr>
<td>Sterile 1.5 ml Centrifuge Tubes</td>
<td>Multiple Vendors</td>
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<tr>
<td>ViaFect Transfection Reagent</td>
<td>Promega</td>
<td>E4981</td>
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* Other transfection reagents may have been tested. Contact CDI’s Technical Support (support@cellulardynamics.com; +1 (877) 320-6888 (US toll-free) or (608) 310-5100) for more information.

Methods

Culturing iCell Cardiomyocytes
1. Thaw and maintain iCell Cardiomyocytes according to the iCell Cardiomyocytes User’s Guide.

   Note: iCell Cardiomyocytes have been transfected successfully at day 7 post-plating; however, other time points may be acceptable. Contact CDI’s Technical Support for more information.

Transfecting iCell Cardiomyocytes
1. On the day of transfection, aspirate the spent medium and replace with fresh iCell Cardiomyocytes Maintenance Medium (Maintenance Medium) at 90% of the culture medium.

   Note: For a 96-well cell culture plate, replace with 0.09 ml/well of medium.

2. Incubate the plate in a cell culture incubator at 37°C, 5% CO₂ for 2 - 4 hours.
3. Prepare a 10X transfection complex solution in Opti-MEM Reduced Serum Medium according to the manufacturer’s instructions.

   **Note:** For a 96-well cell culture plate, prepare 0.01 ml/well of solution.

   **Note:** For ViaFect Transfection Reagent, an optimal reagent (µl):DNA (µg) ratio of 2:1 has been determined for use with iCell Cardiomyocytes.

4. Add the 10X transfection complex solution to the center of each well containing iCell Cardiomyocytes in Maintenance Medium.

   **Note:** It is recommended to rock the plate gently to distribute the transfection complexes evenly across the cell monolayer.

5. Incubate in a cell culture incubator at 37°C, 5% CO₂ overnight.

6. Replace 100% of the medium with fresh Maintenance Medium.

7. Measure transfection efficiency (optional, Figure 1).

   ![Figure 1: iCell Cardiomyocytes Are Transfected with High Efficiency and Low Toxicity Using ViaFect Transfection Reagent](image)

   **Figure 1:** iCell Cardiomyocytes were cultured for 7 days in a 96-well cell culture plate before transfection with a GFP-expressing plasmid DNA (pZsGreen1-N1 VectorGreen, Clontech, Cat. No. 632448) and analyzed at 24 hours post-transfection by (A) fluorescence microscopy. Quantification of cell viability and transfection efficiency was determined at different reagent:DNA ratios using (B) the CellTiter-Glo Luminescence Cell Viability Assay (Promega, Cat. No. G7572) and GFP fluorescence intensity, respectively.

8. Prepare transfected iCell Cardiomyocytes for the desired endpoint assay.

**Summary**

iCell Cardiomyocytes provide a relevant in vitro test system that recapitulates native human cardiac myocyte physiology. Here we describe a protocol for efficiently transfecting foreign DNA in human cardiomyocytes using a liposome-mediated system for assessment of a gene or protein function.
References
