Using Liposome-mediated Transfection for Gene Delivery

Introduction

This protocol describes how to deliver plasmid DNA into iCell® Cardiomyocytes² using the ViaFect Transfection Reagent.

Required Consumables

The following consumables are required in addition to the materials specified in the iCell Cardiomyocytes² User's Guide.

Item	Vendor	Catalog Number
iCell Cardiomyocytes² Kit	Cellular Dynamics International (CDI)	CMC-100-012-000.5 (0.5 unit) CMC-100-012-001 (1 unit)
Opti-MEM Reduced Serum Medium	Life Technologies	31985-062
Plasmid DNA	Multiple Vendors	
Sterile 1.5 ml Centrifuge Tubes	Multiple Vendors	
ViaFect Transfection Reagent*	Promega	E4981

* Other transfection reagents may have been tested. Contact CDI's Technical Support for more information (support@cellulardynamics.com; +1 (877) 320-6688 (US toll-free) or (608) 310-5100).

Methods

Culturing iCell Cardiomyocytes²

1. Thaw and maintain iCell Cardiomyocytes² according to the iCell Cardiomyocytes² User's Guide.

   Note: *iCell Cardiomyocytes² have been transfected successfully at day 2 and 3 post-plating; however, other time points may be acceptable. Contact CDI's Technical Support for more information.*

Transfecting iCell Cardiomycytes²

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 volume.

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

2. 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 4:1 has been determined for use with iCell Cardiomyocytes².*

3. Add the 10X transfection complex solution to the center of each well containing iCell Cardiomyocytes² in Maintenance Medium.

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

4. Incubate in a cell culture incubator at 37°C, 5% CO_2 overnight.

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

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

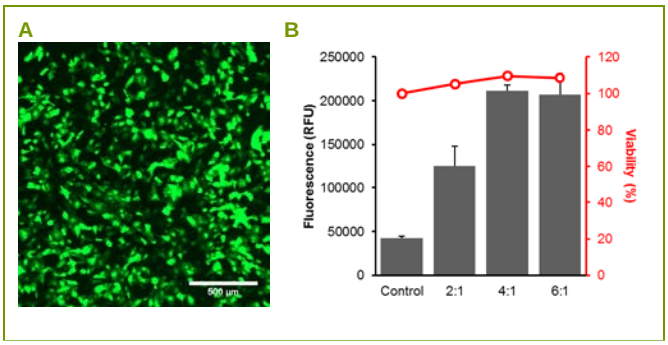

Figure 1: iCell Cardiomyocytes² Are Transfected with High Efficiency and Low Toxicity Using ViaFect Transfection Reagent
iCell Cardiomyocytes² were cultured for 2 days in a 96-well cell culture plate before transfection with a GFP-expressing plasmid DNA (pZsGreen1-N1 VectorGreen, Clontech, Cat. No. 632448) and analyzed at 48 hours post-transfection by (A) fluorescence microscopy. Scale bar = 500 µm. 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.

7. Prepare transfected iCell Cardiomyocytes² for the desired endpoint assay.

Notes

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Summary

iCell Cardiomyocytes² 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.

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