Using Liposome-mediated Transfection for Gene Delivery

Introduction

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

Required Consumables

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

Item	Vendor	Catalog Number
iCell DopaNeurons Kit	Cellular Dynamics International (CDI)	DNC-301-030-001
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

Methods

Culturing iCell DopaNeurons

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

   Note: *iCell DopaNeurons have been transfected successfully at day 4 post-plating: however, other time points may be acceptable. Contact CDI's Technical Support (support@cellulardynamics.com; +1 (877) 320-6688 (US toll-free) or (608) 310-5100) for more information.*

Transfecting iCell DopaNeurons

1. On the day of transfection, aspirate the spent medium and replace with fresh Complete 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. Incubate the plate in a cell culture incubator at 37°C, 5% CO_2 for 2 - 4 hours.

3. Prepare a 10X transfection complex solution in Opti-MEM Reduced Serum Medium according to 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 DopaNeurons.*

4. Add the 10X transfection complex solution to the center of each well containing iCell DopaNeurons in Complete 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% CO_2 overnight.

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

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

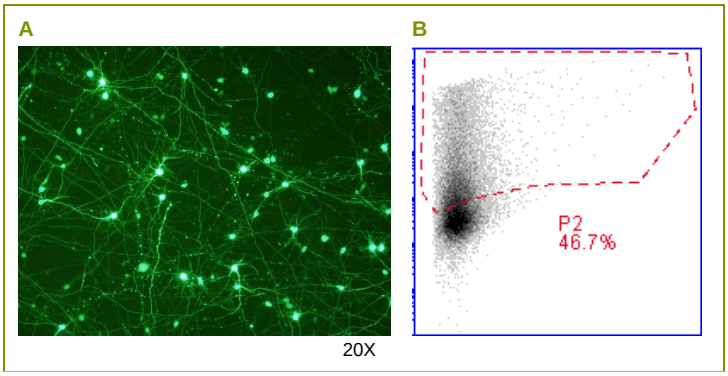

Figure 1: iCell DopaNeurons Are Transfected with High Efficiency and Low Toxicity Using ViaFect Transfection Reagent
iCell DopaNeurons were cultured for 4 days in a 96-well cell culture plate before transfection with a GFP-expressing plasmid DNA (pZsGreen1-N1 VectorGreen, Clontech, Cat. No. 632448) and analyzed at 72 hours post-transfection by (A) fluorescence microscopy or (B) flow cytometry (represented as a percentage of GFP-expressing cells).

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

Summary

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

Notes

Notes

References

1. Cellular Dynamics International, Inc. (2015) iCell Neural Products Application Note: Applying Transfection Technologies to Create Novel Screening Models. www.cellulardynamics.com/lit/.
2. Anson BA. (2015) Building Richer Assays: Transfection of iPSC-derived Tissue Cells Is a Powerful Addition to the Biologist's Tool Box. GEN **35**(2).

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