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, 01279*	Cellular Dynamics International (CDI)	R1032
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

* Formerly known as iCell DopaNeurons Kit (Cat. No. DNC-301-030-001).

Methods

Culturing iCell DopaNeurons

1. Thaw and maintain iCell DopaNeurons according to their 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).

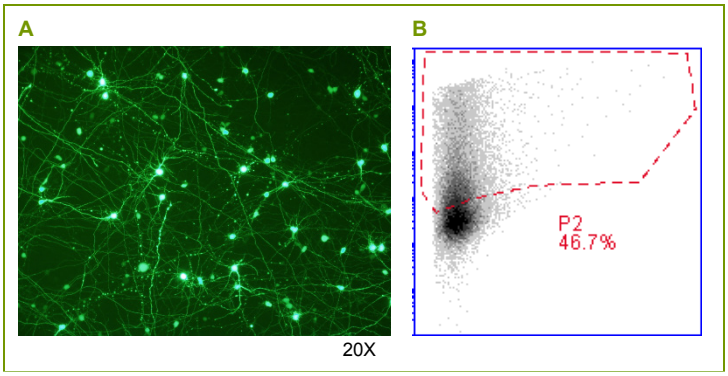

Figure 1: iCell DopaNeurons Are Transfected with High Efficiency and Low Toxicity Using ViaFect Transfection Reagent
iCell DopaNeurons were cultured for 4 days in a 96-well cell culture plate before transfection with a GFP-expressing plasmid DNA (pZsGreen1-N1 VectorGreen, Clontech, Cat. No. 632448) and 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).

Revision History
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