

# Using Liposome-mediated Transfection for Gene Delivery

## Introduction

This Application Protocol describes how to deliver plasmid DNA into iCell® GABANeurons (formerly known as iCell Neurons) using the ViaFect Transfection Reagent.<sup>1, 2</sup>

## Required Consumables

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

Item	Vendor	Catalog Number(s)
iCell GABANeurons Kit, 01279*	Cellular Dynamics International (CDI)	R1011, R1084, R1118
iCell GABANeurons Kit, 01434* <sup>^</sup>	Cellular Dynamics International (CDI)	R1013, R1053
Opti-MEM Reduced Serum Medium	Thermo Fisher Scientific	31985-062
Plasmid DNA	Multiple Vendors	
Sterile 1.5 ml Centrifuge Tubes	Multiple Vendors	
ViaFect Transfection Reagent <sup>+</sup>	Promega	E4981

\* Order the kit whose iCell GABANeurons were derived from the desired donor. iCell GABANeurons, 01279 and iCell GABANeurons, 01434 were derived from apparently healthy, normal donors. iCell GABANeurons, 01434 are exclusive to CDI.

**Note:** This Application Protocol was optimized using iCell GABANeurons Kit, 01434 (Cat. No. R1013). CDI anticipates you will achieve similar results using iCell GABANeurons derived from other apparently healthy, normal donors.

<sup>^</sup> Formerly known as iCell Neurons (Cat. No. NRC-100-010-001).

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

## Methods

### Culturing iCell GABANeurons

Thaw and maintain the neurons according to their User's Guide.

**Note:** iCell GABANeurons have been transfected successfully at day 4 or 21 post-plating; however, other time points may be acceptable. Contact CDI's Technical Support for more information.

### Transfecting iCell GABANeurons

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<sub>2</sub> 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 2:1 has been determined for use with the neurons.

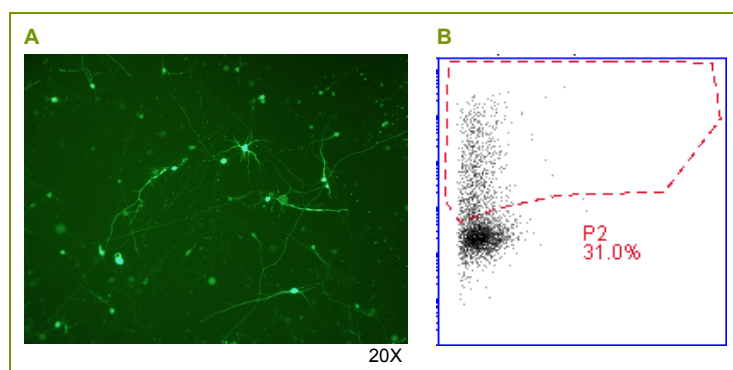
4. Add the 10X transfection complex solution to the center of each well containing the neurons in Complete Maintenance Medium.

**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<sub>2</sub> overnight.

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

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



**Figure 1: iCell GABANeurons Are Transfected with High Efficiency and Low Toxicity Using ViaFect Transfection Reagent**

*iCell GABANeurons, 01434 were cultured for 21 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 the transfected neurons for the desired endpoint assay.

### Summary

iCell GABANeurons 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.

## References

1. Cellular Dynamics International, Inc. (2017) iCell Neural Products Application Note: Applying Transfection Technologies to Create Novel Screening Models. [www.cellulardynamics.com/lit/](http://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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### Revision History

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