Plating into 1536-well Cell Culture Plates

Introduction

The protocol presented here describes how to plate iCell® GABANeurons (formerly known as iCell Neurons) into 1536-well cell culture plates to achieve optimal morphology and functionality.

Required Equipment and Consumables

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

Item	Vendor	Catalog Number(s)
Equipment		
Multichannel Pipettors and Sterile Tips	Multiple Vendors	
Consumables		
iCell GABANeurons Kit, 01279[1]	Cellular Dynamics International (CDI)	R1011, R1084, R1118
iCell GABANeurons Kit, 01434[1, 2]	Cellular Dynamics International (CDI)	R1013, R1053
Poly-D-lysine (PDL) Pre-coated 1536-well Cell Culture Plates	Corning	3836

1 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 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.*

2 Formerly known as iCell Neurons (Cat. No. NRC-100-010-001).

Methods

1. Prepare the Complete Maintenance Medium according to the iCell GABANeurons User's Guide.
2. Thaw iCell GABANeurons according to their User's Guide.
3. Remove a sample of the cell suspension and count the neurons using a hemocytometer. Transfer the cell suspension to a 15 ml centrifuge tube.
4. Concentrate the neurons by centrifuging at 380 x g for 5 minutes.
5. Aspirate the supernatant to 1 ml, being careful not to disturb the pellet.
6. Dilute the 1 mg/ml stock laminin solution in Complete Maintenance Medium to a final concentration of 6.6 µg/ml laminin. Gently mix by inverting the tube.

7. Resuspend the pellet in Complete Maintenance Medium to 1,200,000 cells/ml. Further dilute the cell suspension in equal volume of 6.6 µg/ml laminin solution to achieve a final cell concentration of 600,000 cells/ml with a final laminin concentration of 3.3 µg/ml.

8. Immediately add 5 µl/well of cell suspension into a PDL pre-coated 1536-well cell culture plate (100,000 cells/cm^2). Each well will contain 3,000 cells.

   Note: *Specific applications may require a different seeding density. Contact Technical Support (support@cellulardynamics.com) for guidance.*

9. Pulse the plate at 200 x g for 5 seconds to eliminate bubbles and ensure the cell suspension coats the entire surface of the well.

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

    Note: *Handle the neurons with care to ensure successful adherence. If automation is used for plating cells and exchanging medium, a rate of ~5 µl/second is recommended.*

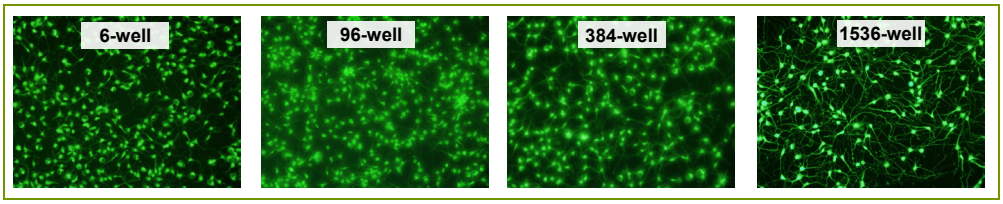

Figure 1: iCell GABANeurons Exhibit Expected Morphology
iCell GABANeurons, 01434 in laminin were plated into PDL pre-coated cell culture plates at 125,000 cells/cm^2 for 6-, 96-, and 384-well formats and 100,000 cells/cm^2 for 1536-well formats. On day 8 post-plating for 6-, 96- and 384-well formats and day 10 post-plating for 1536-well formats, cells were stained with calcein-AM (green, Thermo Fisher Scientific). Scale bar = 50 µm.

Notes

Notes

Summary

iCell GABANeurons provide an in vitro test system that recapitulates native human neuronal characteristics and function. Here we describe a protocol for plating iCell GABANeurons into 1536-well cell culture plates, which enables the use of these cells in high-throughput applications.

Revision History
Version 1.0: April 2018
AP-GABA1536PL180418