Measuring Synchronous Neuronal Activity on the Maestro Multielectrode Array

Introduction

iCell® DopaNeurons, human induced pluripotent stem cell (iPSC)-derived neurons, exhibit biochemical, electrophysiological, and pathophysiological properties characteristic of native human neurons. Due to their high purity, functional relevance, and ease of use, iCell DopaNeurons represent an optimal in vitro test system for neurobiology interrogations in basic research and many areas of drug development.

Axion BioSystems' Maestro multielectrode array (MEA) technology is a non-invasive, label-free platform that measures the electrical activity of single cells or cellular networks. With proper handling, iCell DopaNeurons can be thawed and cultured directly on MEAs to form neuronal networks amenable to electrophysiological interrogation.

This Application Protocol describes how to thaw, plate, and culture iCell DopaNeurons on the Maestro MEA system and provides basic instructions for data acquisition and analysis.

Required Equipment, Consumables, and Software

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

Item	Vendor	Catalog Number
Equipment		
12-channel Pipettor, 200 µl	Multiple Vendors	
Maestro MEA System	Axion BioSystems	
Consumables		
iCell DopaNeurons Kit, 01279[1]	Cellular Dynamics International (CDI)	R1032
iCell Neural Supplement B[2]	Cellular Dynamics International (CDI)	M1029
iCell Nervous System Supplement[3]	Cellular Dynamics International (CDI)	M1031
0.22 µm Sterile Filter Unit	Multiple Vendors	
48-well MEA Plate[4]	Axion BioSystems	M768-KAP-48
Borate Buffer, 20X	ThermoFisher Scientific	28341
BrainPhys Neuronal Medium	STEMCELL Technologies	05790
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	ThermoFisher Scientific	14190
Laminin	Sigma	L2020
MicroClime Environmental Plate Lid (MicroClime Lid)	LabCyte	LLS-0310-IP

Item	Vendor	Catalog Number
N-2 Supplement, 100X	ThermoFisher Scientific	17502-048
Penicillin-streptomycin, 100X	ThermoFisher Scientific	15140-122
Poly(ethyleneimine) (PEI) Solution, 50%, Average M_w ~750K	Sigma	181978-100G
Sterile 1.5 ml, 15 ml, and 50 ml Centrifuge Tubes	Multiple Vendors	
Sterile Tissue Culture Grade Distilled Water (Sterile Water)	Multiple Vendors	
Software		
Axion Integrated Studio (AxIS, version 2.1 or higher)[4]	Axion BioSystems	

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

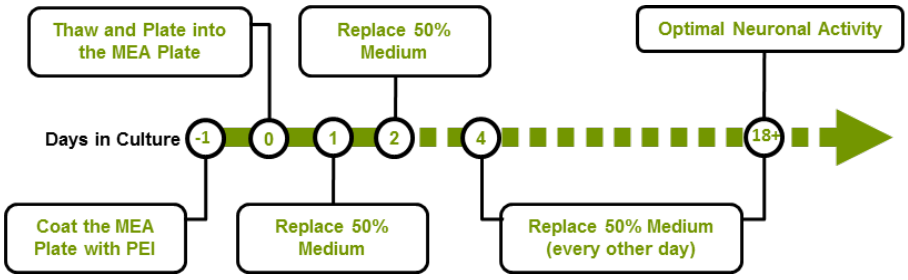
2 Formerly known as iCell DopaNeurons Medium Supplement (Cat. No. DNM-301-031-001).

3 Formerly assigned Cat. No. NSS-301-031-001.

4 This Application Protocol provides instructions for using 48-well MEA plates. Contact CDI's Technical Support (support@cellulardynamics.com; +1 (877) 320-6688 (US toll-free) or (608) 310-5100) for instructions for using other plate formats.

Workflow

iCell DopaNeurons are thawed and plated into a 48-well MEA plate pre-coated with PEI and laminin. On day 1 post-plating, 100% of spent medium is replaced with fresh medium. Every 2 - 3 days thereafter, 50% medium changes are performed. Optimal synchronous neuronal activity is observed from day 18 post-plating.



Methods

When planning for experiments, be aware one unit of ≥5 million iCell DopaNeurons fill at least two 48-well MEA plates. Prepare reagents, plates, and medium accordingly.

Preparing the PEI Solution

Due to the high viscosity of the 50% PEI solution, you prepare an intermediate 7% PEI solution that is further diluted to a final concentration of ~0.07%.

1. Prepare 100 ml of 1X borate buffer by diluting 5 ml of 20X borate buffer in 95 ml distilled water.
2. Prepare an intermediate ~7% PEI solution by pouring 1 ml of 50% PEI solution into a 15 ml centrifuge tube and allow to settle. Add 6 ml of 1X borate buffer to obtain an intermediate ~7% solution.

   Note: *Vortexing is recommended to get the viscous PEI into solution.*

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3. Prepare a final ~0.07% PEI solution by diluting 500 µl of intermediate ~7% PEI solution in 49.5 ml 1X borate buffer. Filter through a 0.22 µm filter unit.

   Note: *The final ~0.07% PEI solution can be stored at 4°C for only 1 week. Fresh 0.07% PEI solution should be made each week for use.*

Preparing the 48-well MEA Plate

1. Add 80 µl/well of ~0.07% PEI solution to the 48-well MEA plate directly covering electrodes at the center of each well. Incubate at 37°C for 1 hour.
2. Aspirate the PEI solution from the 48-well MEA plate. Do not allow the wells to dry.
3. Immediately rinse twice with ≥300 µl/well of sterile D-PBS. Rinse once more with ≥300 µl/well of sterile water and air-dry the 48-well MEA plate with the lid removed in a sterile biological safety cabinet overnight.

   Note: *It is critical to allow the MEA plate to air-dry overnight to achieve optimal cell attachment and maximal performance.*

   Note: *To avoid damaging the MEA plate, do not turn on the UV light in the biological safety cabinet while air-drying the plate.*

Thawing iCell DopaNeurons

The following procedure describes how to thaw 1 vial of iCell DopaNeurons into two 48-well MEA plates. Do not prepare more than two 48-well MEA plates at one time.

1. Prepare 100 ml of complete BrainPhys medium by adding the following components:

Component	Amount (ml)	Final Concentration
BrainPhys Neuronal Medium	95	Not Applicable
iCell Neural Supplement B	2	Not Applicable
iCell Nervous System Supplement	1	Not Applicable
Laminin, 1 mg/ml	0.1	1 µg/ml
N-2 Supplement, 100X	1	1X
Penicillin-streptomycin, 100X	1	1X

2. Filter through a 0.22 µm filter unit.

   Note: *Store the complete BrainPhys medium at 4°C for up to 2 weeks.*

3. Prepare 80 µg/ml laminin pre-dot solution by diluting 80 µl of 1 mg/ml stock laminin solution in 1 ml of complete BrainPhys medium in a 1.5 ml centrifuge tube.

   Note: *Thaw stock laminin solution at room temperature or at 4°C overnight. Do not thaw the stock laminin solution in a 37°C water bath. Do not vortex the stock laminin solution.*

4. Tilt the 48-well MEA plate pre-coated with PEI solution at a 30 degree angle so that the bottom of all wells are visible (Figure 1). Pipette a 10 µl/well droplet of 80 µg/ml laminin pre-dot solution directly over the recording electrode area of each well. Incubate the 48-well MEA plate at 37°C for 30 minutes.

5. Prepare 10 µg/ml laminin solution by diluting 250 µl of 1 mg/ml stock laminin solution in 25 ml of complete BrainPhys medium. Gently mix by inverting the tube.
6. Thaw the neurons according to their User's Guide in a 50 ml centrifuge tube and dilute the cell suspension to a final volume of 10 ml in complete BrainPhys medium containing 10 µg/ml laminin.
7. Remove a sample of the cell suspension and count the neurons using a hemocytometer to verify the viability and total number of cells listed in the Certificate of Analysis.

   Note: *With proper cell handling, expect to recover within ~20% of the total cell number and viability listed in the Certificate of Analysis.*
8. Transfer the cell suspension to a 15 ml centrifuge tube and concentrate the cells by centrifuging at 380 x g for 5 minutes.
9. Aspirate the supernatant to just above the cell pellet, leaving approximately 50 µl, being careful not to disturb the pellet.

   Note: *This volume approximation is provided due to the imprecise nature of vacuum aspiration.*
10. Measure the total volume of the cell suspension with a pipettor. Add the dotting medium to the cell suspension to reach a concentration of 8 million cells/ml.
11. Transfer the cell suspension from the 15 ml centrifuge tube to a 1.5 ml centrifuge tube.

    Note: *The final cell suspension at 8,000,000 cells/ml corresponds to plating 80,000 cells/well in the MEA plate.*

Plating iCell DopaNeurons into the 48-well MEA Plate

1. Thoroughly mix the cell suspension by gently inverting the tube 2 - 3 times. Tilt the 48-well MEA plate at a 30 degree angle so that the bottom of all wells are visible. Immediately dispense a 10 µl/well droplet of the cell suspension directly into the 10 µl/well droplet of laminin pre-dot solution, resulting in a final droplet size of 20 µl/well (Figure 1).

   Note: *CDI recommends dispensing one row at a time and mixing the cell suspension by gently tapping the tube between each of the 6 rows to ensure an even distribution of the cell suspension.*

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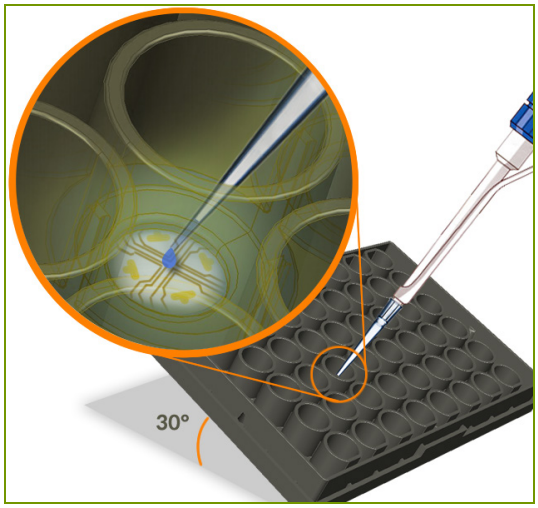

Figure 1: Example Droplet Placement
Tilt the 48-well MEA plate 30 degrees and dispense a 10 µl droplet of cell suspension into the 10 µl droplet of laminin pre-dot solution located in the center of the well over the recording electrode area.

2. Add approximately 2 ml of sterile water to the area surrounding the wells of the 48-well MEA plate to prevent droplet evaporation. Do not allow water into the wells of the 48-well MEA plate.

   Note: *CDI recommends adding the water after plating the cell suspension to avoid water leaking into wells when the 48-well MEA plate is tilted.*

3. Cover the 48-well MEA plate with a sterile, hydrated MicroClime lid and incubate in a cell culture incubator at 37°C, 5% CO_2, 95% humidity for 60 minutes.

   Note: *Hydrate the lid with water according to manufacturer's recommendations.*

4. Before adding medium, load a 12-channel pipettor with sterile tips according Figure 2.

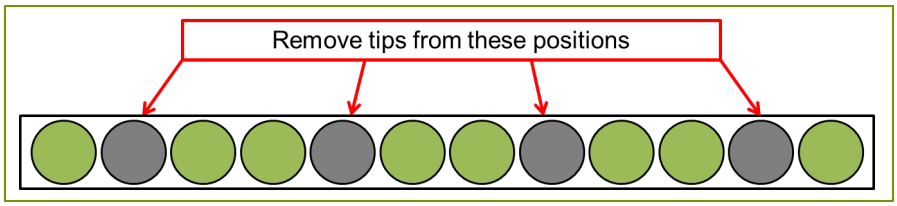

Figure 2: Tip Loading Strategy on a 12-channel Pipettor
A 12-channel pipettor loaded with sterile tips arranged in the highlighted positions (green) is suitable for medium addition to a column of a 48-well MEA plate.

5. Tilt the 48-well MEA plate at an ~75 degree angle. Gently add 150 µl/well of complete BrainPhys medium containing 10 µg/ml laminin down the side of the well of the plate one row at a time using the 12-channel pipettor. Adding the medium too quickly will dislodge the adhered neurons.

   Note: *Timing is critical in this step. The performance is compromised if the droplets are allowed to dry. CDI recommends adding a small volume of medium to all wells first rather than adding the total volume in each well at once.*

6. Slowly return the 48-well MEA plate to a flat position on the surface of the biological safety cabinet to allow the complete BrainPhys medium to gently cover the droplet.
7. Add an additional 150 µl/well of complete BrainPhys medium to the side of the well to reach a final volume of 300 µl/well.
8. Cover the 48-well MEA plate with the MicroClime lid and incubate in a cell culture incubator at 37°C, 5% CO_2, 95% humidity.

Maintaining iCell DopaNeurons on the 48-well MEA Plate

1. On day 1 post-plating, equilibrate an aliquot (approximately 15 ml for a 48-well MEA plate) of complete BrainPhys medium to room temperature.
2. Load a 12-channel pipettor with sterile tips as identified in Figure 2 and remove 50% of the spent medium from the 48-well MEA plate one row at a time.
3. Gently add 150 µl/well of the room temperature complete BrainPhys medium to the side of the well of the 48-well MEA plate one row at a time using the 12-channel pipettor. Adding the medium too quickly may dislodge adhered cells.
4. Cover the 48-well MEA plate with the MicroClime lid and incubate in a cell culture incubator at 37°C, 5% CO_2, 95% humidity.
5. Repeat the 50% medium exchange on day 2 post-plating.
6. Perform a 50% medium replacement every 2 - 3 days by removing 150 µl/well of the spent complete BrainPhys medium then adding 150 µl/well of fresh medium.

   Optimal synchronous neuronal activity is observed at approximately day 18 post-plating.

Data Acquisition and Analysis

Electrical activity on the Maestro MEA system is acquired and analyzed using the AxIS Software according to the manufacturer's guidelines.

1. On the day of a recording, change 50% medium approximately 2 - 4 hours before data acquisition.
2. Set the temperature of the instrument to 37°C and use environmental control for at least 5 - 10 minutes before recording a measurement.

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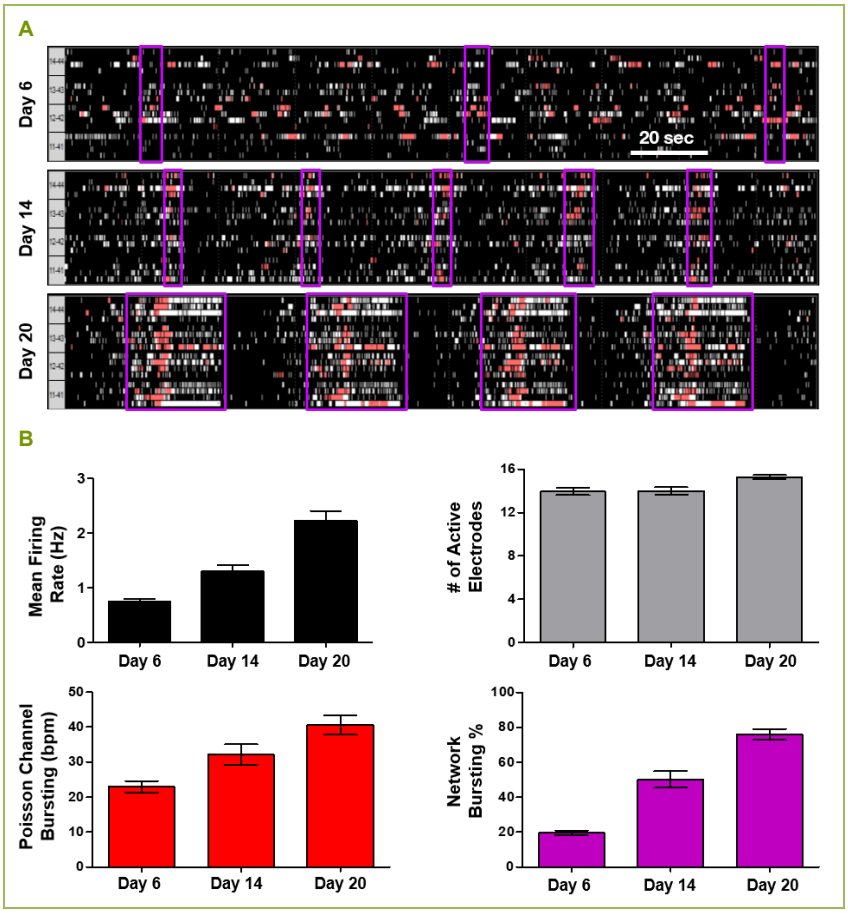

Figure 3: iCell DopaNeurons Develop Synchronous Network Activity over Time
iCell DopaNeurons cultured in complete BrainPhys medium exhibit high levels of neuronal activity as soon as day 2 post-plating and routinely incorporate ≥12 of 16 active electrodes/well of the MEA plate. Importantly, these cells display network-wide bursts in each well with optimal synchronous neuronal activity observed from day 18 post-plating. (A) As shown in the raster plots, where the white lines indicate spikes or neuronal action potentials, Poisson channel bursting is observed during the first week post-plating (day 6) as indicated by the red tick marks. This bursting rate (bursts per minute, bpm) increases over time (day 14), and eventually the neuronal activity develops into a synchronized network that organizes the entire culture (day 20). The purple boxes around the organized spikes in each raster plot denote inter-spike interval (ISI) network bursts captured by the AxIS Software. The network bursting percentage is defined as the number of spikes that fall within the purple box divided by the total number of tick marks measured during the recording. The higher this percentage is, the more synchronous the neuronal culture is considered to be. (B) The graphs represent mean and SEM values calculated on days 6, 14, and 20 post-plating.

Summary

iCell DopaNeurons can be thawed and cultured directly on MEA plates where spontaneous electrical signals can be monitored and neuronal networks established. These cells are a mix of excitatory and inhibitory neurons with the ability to form synchronous networks. The methods and data presented here highlight the ease of using iCell DopaNeurons on the Maestro MEA system.

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Revision History
Version 1.3: February 2018
AP- DNCAXN180226