

Measuring Neuronal Activity: *Extracellular Single-unit Recordings on the Maestro Multielectrode Array*

Introduction

iCell® Neurons are human induced pluripotent stem cell (iPSC)-derived neurons that exhibit biochemical, electrophysiological, and pathophysiological properties characteristic of native human neurons. Due to their high purity, functional relevance, and ease of use, iCell Neurons 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 network. With proper handling, iCell Neurons can be thawed and cultured directly on MEAs to form neuronal networks amenable to electrophysiological interrogation. iCell NeuroAnalyzer, a MATLAB-based script, analyzes the neuronal electrical activity detected on the Maestro MEA system. Together, iCell Neurons, Axion Biosystems' MEA system, and iCell NeuroAnalyzer provide an excellent, non-invasive platform for assessing the potential effects of compound modulation of neurotransmission.

This Application Protocol describes how to handle iCell Neurons for use on the Maestro MEA system and provides basic instructions for compound treatments, data acquisition, and analysis using the iCell NeuroAnalyzer.

Required Equipment, Consumables, and Software

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

Item	Vendor	Catalog Number
Equipment		
Maestro Multielectrode Array (MEA) System	Axion BioSystems	
12-channel Pipettor (20 and 200 µl)	Multiple Vendors	
Consumables		
iCell Neurons Kit	Cellular Dynamics International (CDI)	NRC-100-010-001
0.22 µm Filter	Multiple Vendors	
1X Neurobasal-A (NBA) Medium	Life Technologies	10888-022
48-well Multielectrode Array (MEA) Plate ¹	Axion BioSystems	M768-KAP-48
50% Polyethyleneimine (PEI) Solution	Sigma	P3143-100ml
Boric Acid	Fisher	A73-500
KnockOut Serum Replacement (KSR)	Life Technologies	10828-028

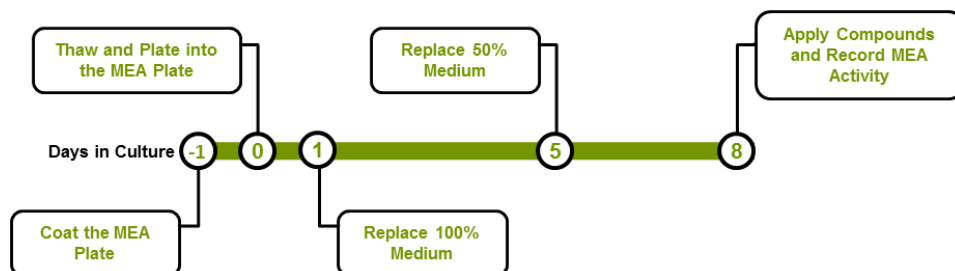
Item	Vendor	Catalog Number
Laminin	Sigma	L2020
MicroClimate Environmental Plate Lid	LabCyte	LLS-0310-IP
Penicillin-streptomycin (100X)	Life Technologies	15140-122
Sodium Tetraborate	Sigma	221732-100g
Sterile 1.5 ml Centrifuge Tubes	Multiple Vendors	
Sterile Water	Multiple Vendors	
Software		
CDNeuronconfig.datastream ²	Cellular Dynamics International (CDI)	
iCell NeuroAnalyzer (version 1.0) ²	Cellular Dynamics International (CDI)	
Axion Integrated Studio (AxIS) (version 1.8 or later)	Axion BioSystems	
MATLAB (R2012b, version 8.0, or later)	MathWorks	

¹ 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.

² Contact CDI Technical Support for CDNeuronconfig.datastream and iCell NeuroAnalyzer files.

Workflow

iCell Neurons are thawed and plated into a 48-well MEA plate pre-coated with PEI solution. On day 1 post-plating, 100% of spent medium is replaced with NBA + 10% KSR. On day 5 post-plating, 50% of spent medium is replaced with NBA + 10% KSR. On day 8 post-plating, baseline activity is recorded, cells are treated with compounds, and the activity is subsequently recorded.



Methods

Preparing the 48-well MEA Plate

1. Prepare 1 l of borate buffer by dissolving 3.10 g of boric acid and 4.75 g of sodium tetraborate in distilled water. Adjust the pH to 8.4. Scale volumes, if necessary.
2. Prepare a 0.05 - 0.1% PEI solution by diluting 50% PEI solution in borate buffer. Filter the 0.05 - 0.1% PEI solution through a 0.22 μ m filter.
Note: 0.05 - 0.1% PEI solution can be stored at 4°C for up to 1 month.
3. Add 125 μ l/well of 0.05 - 0.1% PEI solution to the 48-well MEA plate. Incubate at room temperature for 1 hour.

4. Aspirate the PEI solution from the 48-well MEA plate. Do not allow the wells to dry.
5. Rinse 4 times with at least 300 μ l/well of sterile water.
6. Air-dry the 48-well MEA plate with the lid off in a sterile biological safety cabinet overnight.

Note: *It is essential to allow the 48-well MEA plate to air-dry overnight to achieve optimal cell attachment and maximal performance.*

Thawing iCell Neurons

The following procedure details thawing 1 vial of iCell Neurons into a 48-well MEA plate. Scale volumes accordingly for 2 vials of iCell Neurons. Do not prepare more than two 48-well MEA plates at one time.

1. Prepare Complete iCell Neurons Maintenance Medium (Complete Maintenance Medium) according to the iCell Neurons User's Guide.

Optional: *Penicillin-streptomycin can be added to Complete Maintenance Medium at 1X final concentration.*

2. Dilute the stock laminin solution (1 mg/ml) by adding 250 μ l laminin into 25 ml Complete Maintenance Medium to a final concentration of 10 μ g/ml. Gently mix by inverting the tube.

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

3. Thaw iCell Neurons according to the User's Guide and dilute the cell suspension to a final volume of 10 ml in Complete Maintenance Medium containing 10 μ g/ml laminin.
4. (Optional) Remove a sample of the cell suspension and count the neurons using a hemocytometer to determine the viability and total number of cells.
5. Transfer the cell suspension to a 15 ml centrifuge tube.
6. Concentrate the neurons by centrifuging at 380 x g for 5 minutes.
7. Aspirate the supernatant to just above the cell pellet, leaving approximately 50 μ l, being careful not to disturb the pellet.

Note: *This volume approximation is provided due to the imprecise nature of vacuum aspiration.*

8. Add 125 μ l of Complete Maintenance Medium containing 10 μ g/ml laminin to the cell pellet and resuspend gently by pipetting up and down.
9. Measure the total volume of cell suspension with a pipettor. Add Complete Maintenance Medium containing 10 μ g/ml laminin to reach a final volume of 220 μ l. Mix by gently pipetting.
10. Transfer the cell suspension to a 1.5 ml centrifuge tube.

Plating iCell Neurons 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 an angle so that the bottom of all wells are visible. Immediately dispense a 4 μ l/well droplet of the cell suspension directly over the recording electrode area of the well of the 48-well MEA pre-coated with PEI solution (Figure 1).

Note: CDI recommends dispensing 1 row at a time and mixing the cell suspension between each of the 6 rows to ensure an even distribution of the cell suspension.

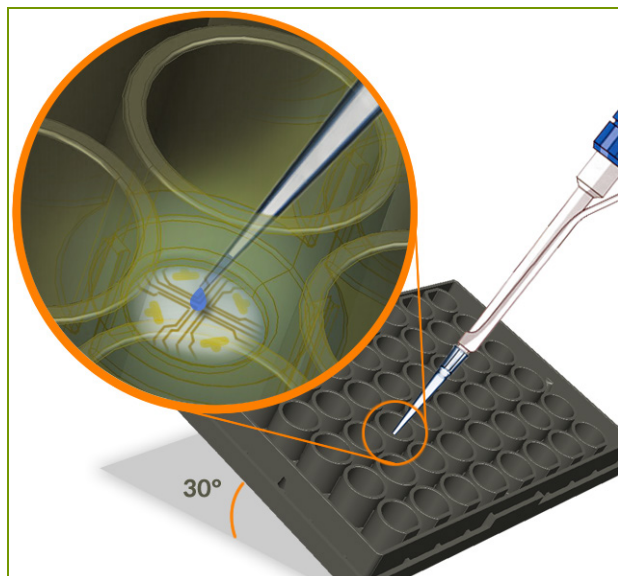


Figure 1: Example Droplet Placement

Tilt the 48-well MEA plate 30 degrees and dispense a 4 μ l droplet of cell suspension over the recording electrode area of each well.

2. Add 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 plate is tilted.

Note: The exact volume of water is not critical as long as the 48-well MEA plate maintains a moist environment.

3. Cover the 48-well MEA plate with a sterile MicroClime Environmental lid and incubate in a cell culture incubator at 37°C, 5% CO₂, 95% humidity for 40 minutes.

Note: Do not allow the droplets to incubate in the 48-well MEA plate for longer than 45 minutes.

4. Before adding medium, load a 12-channel pipettor with sterile tips and remove tips from the positions identified in Figure 2.

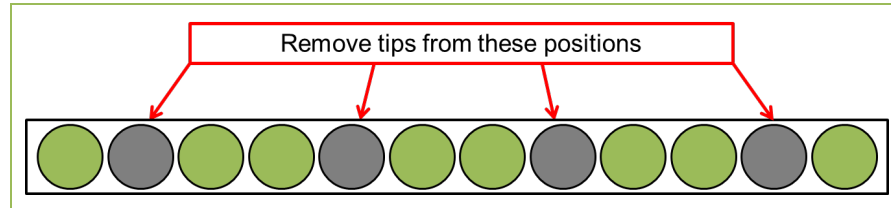


Figure 2: Tip Loading Strategy on a 12-channel Pipettor

A 12-channel pipettor loaded with sterile tips arranged in the highlighted positions (green) is suitable for medium addition to a 48-well MEA plate.

5. Tilt the plate at a steep angle (~75 - 80 degrees). Gently add 150 μl /well of Complete Maintenance Medium containing 10 $\mu\text{g}/\text{ml}$ laminin down 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 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 medium gently to cover the droplet.
7. Repeat step 5 to reach a final volume of 300 μl /well.
8. Cover the 48-well MEA plate with a sterile MicroClime Environmental lid and incubate in a cell culture incubator at 37°C, 5% CO₂, 95% humidity.

Maintaining iCell Neurons on the 48-well MEA Plate

1. Prepare NBA medium supplemented with 10% KSR and 1X penicillin-streptomycin (NBA + KSR medium). Filter through a 0.22 μm filter.

Optional: Penicillin-streptomycin can be added to the medium at 1X final concentration.

2. On day 1 post-plating, equilibrate the NBA+KSR medium to 37°C in a water bath.
3. Load a 12-channel pipettor with sterile tips as identified in Figure 2 and remove the spent medium from the 48-well MEA plate one row at a time.
4. Gently add 150 μl /well of 37°C NBA+KSR 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 will dislodge the adhered neurons.
5. Repeat step 4 to reach a final volume of 300 μl /well.
6. Cover the 48-well MEA plate with a sterile MicroClime Environmental lid and incubate in a cell culture incubator at 37°C, 5% CO₂, 95% humidity for 4 days.
7. On day 5 post-plating, replace 50% of spent medium (150 μl /well) with 150 μl /well of fresh 37°C NBA+KSR medium.
8. Incubate in a cell culture incubator at 37°C, 5% CO₂, 95% humidity for 3 days.

Note: For optimal performance, perform data acquisition on day 8 post-plating.

Note: Do not replace medium the day of the assay.

Data Acquisition

At day 8 post-plating, the neuronal preparation is suitable for data acquisition. Data acquisition consists of a pre-application recording (baseline), a compound application recording, followed by a post-application recording (dose).

Electrical activity is acquired using the AxIS Software. The `CDIneuronconfig.datastream` file ensures the appropriate data acquisition settings are loaded into the AxIS Software.

Setting up AxIS Software

1. Right-click the *MAESTRO* icon beneath the Streams window.
2. Select *Configuration -> Load* and navigate to the *CDIneuronconfig.datastream* file.
3. Click the *Schedule Recordings Setup* icon on the AxIS dashboard and enter the following settings:

Parameter	Setting
<i>Record Every</i>	<i>4.05 minutes</i>
<i>Record For</i>	<i>4 minutes</i>
<i>Execute</i>	<i>Exactly 3 Times</i>
<i>Starting</i>	<i>Immediately</i>

4. Click the *Experimental Setup Properties* icon on the AxIS dashboard.
5. Select raw data (.raw) and AxIS Spikes (.spk) data streams to record.

Recording and Applying Compounds

1. Click the *Play* button and wait for activity to display on the heat map.
2. Click the *Schedule Recordings Setup* icon on the AxIS dashboard.
3. Click the **Start Schedule** button to initiate the baseline recording.
4. Remove the MicroClime Environmental lid after the first 4-minute recording is complete, leaving the plate running on the amplifier.
5. Quickly add 10 μ l/well of compound or control solution to the appropriate wells. Apply the same compound and concentration to the wells within a column.

Note: *It is important to minimize the time without the MicroClime Environmental lid. Compound application should be completed in <90 seconds.*

6. Leaving the plate on the amplifier, cover the MEA with the MicroClime Environmental lid and continue the dose recording without interruption.

Data Analysis

During data acquisition, AxIS Software generates a .spk file. Data analysis is performed on this file using iCell NeuroAnalyzer, an analysis script, in MATLAB. iCell NeuroAnalyzer is designed to allow for 8 different conditions or treatments (n = 6) per plate. All wells within a column are averaged during analysis and should contain the same treatment and concentration of compound.

Analyzing Neuronal Activity

1. (First time use only) Install iCell NeuroAnalyzer in MATLAB.
2. Click *iCell NeuroAnalyzer* icon in the MATLAB App tab.
3. Perform data analysis according to the instructions on the screen.

Note: When prompted to enter group labels, label control columns as “ctrl.” All columns labeled “ctrl” are pooled together and listed as a “pControl” group. The title entered during the prompt is the name of a .txt file containing the data analysis results.

Once analysis completes, the graph in Figure 3 appears on-screen. Graph values are saved in a .txt file.

Output of iCell NeuroAnalyzer

iCell NeuroAnalyzer generates a folder within the same location of the analyzed .spk file and titled “Analysis” combined with the name of the .spk file. This folder contains:

- Raster plots for each electrode and well (figures and MATLAB files)
- Burst detection plots for each well (figures and MATLAB files)
- Mean firing and bursting rate statistics for each recording (figures and Results_v4 file)
- Comparison of baseline and dose recording statistics (figures and .txt files)

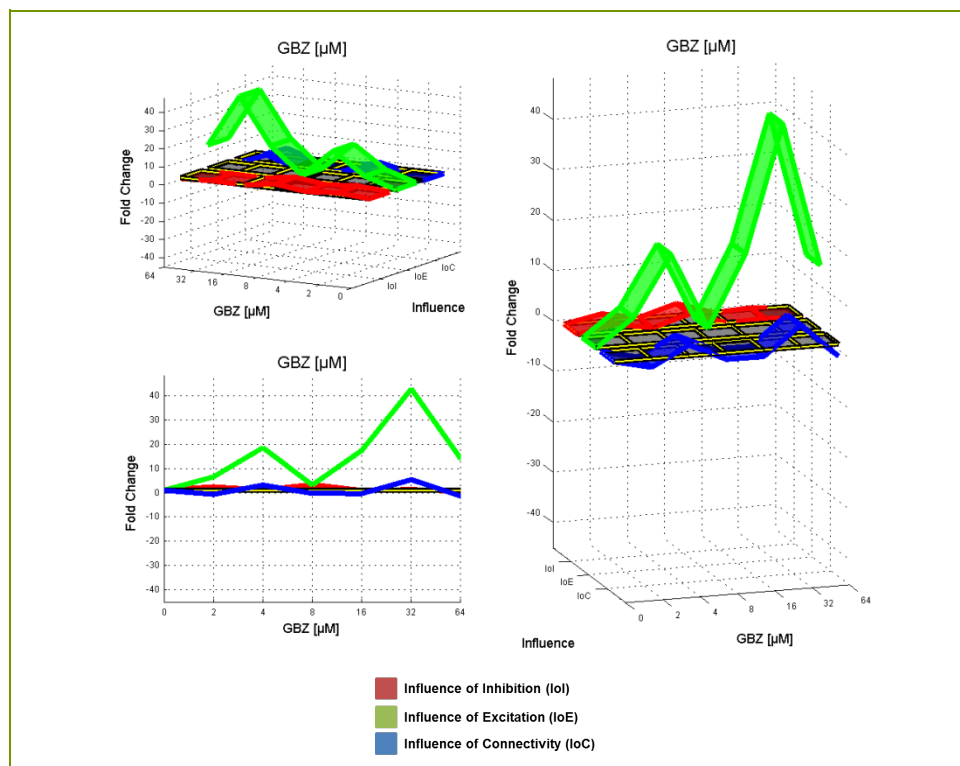


Figure 3: Quantitative Effects of GABA_A Receptor Modulation on the Electrical Activity of iCell Neurons

Blocking all GABA_A receptors with GABAzine (GBZ) produced the expected results on the firing rate (influence of inhibition), bursting rate (influence of excitation), and bursting intensity (influence of connectivity) of the neuronal network. Treatments are compared to an untreated pooled control (pControl). Measures are presented as fold changes compared to baseline after drug treatment (mean ± SEM).

Summary

iCell Neurons can be thawed and cultured directly on MEAs where neuronal networks are established and the spontaneous electrical signals can be monitored. The neuronal electrical activity can be modulated with compounds and functional responses can be robustly quantified. The methods and data presented here highlight the ease of using iCell Neurons on the Maestro MEA system. Together, these products offer a higher throughput in vitro system for gathering relevant data on the electrophysiological activity of human neuronal cells.

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