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iCell® GlutaNeurons
Application Protocol

Measuring Neuronal Activity:
Maestro Multielectrode Array

Introduction

iCell® GlutaNeurons, human glutamatergic cortical neurons derived from induced pluripotent stem (iPS) cells, exhibit typical physiological characteristics and form functional neuronal networks. Due to their high purity, functional relevance, and ease of use, iCell GlutaNeurons represent an optimal in vitro test system that is suitable for use in targeted drug discovery, toxicity testing, and other life science research.

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 GlutaNeurons 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 GlutaNeurons 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 GlutaNeurons User's Guide.

Item	Vendor	Catalog Number
Equipment		
12-channel Pipettor, 200 µl	Multiple Vendors	
Maestro Multielectrode Array (MEA) System	Axion Biosystems	
Consumables		
iCell GlutaNeurons Kit, 01279[1]	Cellular Dynamics International	R1034
iCell Neural Supplement B[2]	Cellular Dynamics International	M1029
iCell Nervous System Supplement[3]	Cellular Dynamics International	M1031
1 ml Pipette	Multiple Vendors	
10 ml Serological Pipette	Multiple Vendors	
20 µl Pipette	Multiple Vendors	
48-well Multielectrode Array (MEA) Plate[2]	Axion Biosystems	M768-KAP-48
50% Polyethyleneimine (PEI) Solution	Sigma	181978-100g
Borate Buffer, 20X	Thermo Fisher Scientific	28341
Laminin	Sigma-Aldrich	L2020
MicroClime Environmental Plate Lid (MicroClime Lid)	LabCyte	LLS-0310-IP

Item	Vendor	Catalog Number
Sterile 1.5 ml Centrifuge Tubes	Multiple Vendors	
Sterile Tissue Culture Grade Distilled Water (Sterile Water)	Multiple Vendors	
Software		
Axion Integrated Studio (AxIS, version 2.3 or higher)	Axion Biosystems	

1 Formerly known as iCell GlutaNeurons (Cat. No. GNC-301-030-001).

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

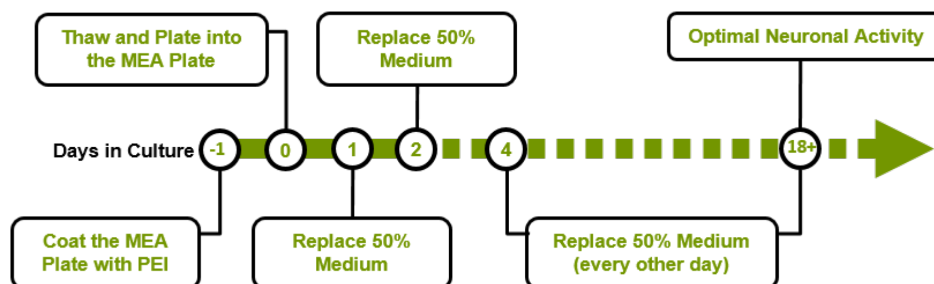
3 Formerly assigned Cat. No. NSS-301-031-001.

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.

Notes

Workflow

iCell GlutaNeurons are thawed into complete BrainPhys medium and plated in complete BrainPhys medium plus laminin (dotting medium) into a 48-well MEA plate pre-coated with PEI solution. On day 1 post-plating, 50% of spent medium is replaced with complete BrainPhys medium. A 50% medium change is performed on day 2 post-plating and then every other day with complete BrainPhys medium. Synchronous neural activity can be detected starting around day 12 and may continue for ≥30 days.



Methods

Preparing the PEI Solution

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.*
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.*

Notes

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.*

Media Preparation

1. Prepare the complete BrainPhys medium according to the iCell GlutaNeurons User's Guide.

   Note: *The complete BrainPhys medium can be stored at 4°C for 14 days. Given the experimental workflow, additional medium will need to be prepared.*

2. Prepare the dotting medium with sterile-filtered complete BrainPhys medium and laminin using the volumes indicated in the table below.

Dotting Medium Components	Volume	Final Concentration
Complete BrainPhys Medium	900 µl	Not Applicable
Laminin, 1 mg/ml	100 µl	100 µg/ml

Thawing iCell GlutaNeurons

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

1. Equilibrate the complete BrainPhys Medium to room temperature for >15 minutes.
2. Thaw iCell GlutaNeurons 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.
3. Remove a sample of the cell suspension and count iCell GlutaNeurons using a hemocytometer to verify the viability and total number of cells listed in the Certificate of Analysis.

   Note: *With proper handling, expect to recover within ~20% of the total cell number and viability listed in the Certificate of Analysis.*

4. Concentrate iCell GlutaNeurons by centrifuging at 400 x g for 5 minutes.
5. Aspirate the supernatant to just above the cell pellet, leaving ~50 µl, being careful not to disturb the pellet.

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

6. Measure the total volume of the cell suspension with a pipettor. Add the dotting medium to the cell suspension to reach a concentration of 12 million cells/ml.
7. Transfer the cell suspension to a 1.5 ml centrifuge tube.

Plating iCell GlutaNeurons 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 10 µl/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.*

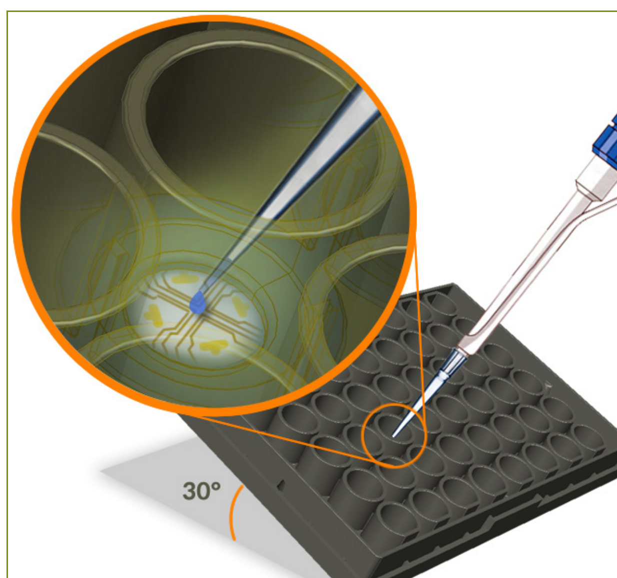


Figure 1: Example Droplet Placement
Tilt the 48-well MEA plate 30 degrees and dispense a 10 µl droplet of cell suspension over the recording electrode area of each well.

2. Add ~1 ml of sterile water to each end of the 48-well MEA plate to prevent droplet evaporation. Do not allow water into the wells of the 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: *Do not allow the droplets to incubate in the 48-well MEA plate for longer than 75 minutes.*

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

Notes

Notes

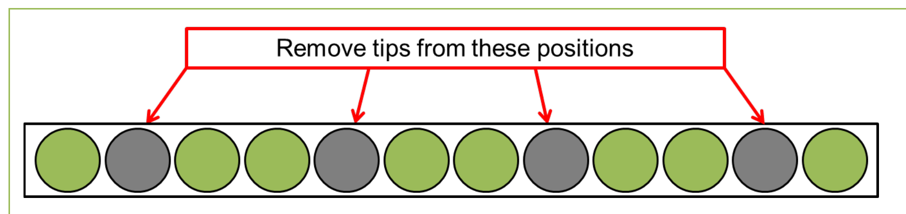


Figure 2: Tip Loading Strategy on a 12-channel Pipettor
A 12-channel pipettor loaded with sterile tips arranged in the highlighted positions (green) is suitable for medium addition to a 48-well MEA plate.

5. Tilt the plate at a steep angle (~75 - 80°). Gently and slowly add (~5 seconds add time) 150 µl/well of the complete BrainPhys medium down the side of the well of the 48-well MEA plate one row at a time using the 12-channel pipettor. Repeat this step to a final volume of 300 ml/well. Adding the medium too quickly will dislodge the adhered neurons.
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. 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 GlutaNeurons on the 48-well MEA Plate

1. On day 1 post-plating, equilibrate the 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 (150 µl) from each well of the 48-well MEA plate, 3 rows at a time.
3. Gently and slowly add (~5 seconds add time) 150 µl/well of the 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 will dislodge the adhered neurons.
4. Cover the 48-well MEA plate with the MicroClime lid. Incubate in a cell culture incubator at 37°*C*, 5% CO_2, 95% humidity for 1 day.
5. Repeat the 50% medium exchange on day 2 post-plating.
6. Perform a 50% medium exchange every other day.

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. Neural activity can be detected starting on day 4 post-plating and synchronous firing from a macro network can be detected at approximately day 12 post-plating. The optimal performance for synchronous network activity can be seen on days 18 - 24 post-plating.

1. On the day of a recording, replace 50% of the spent medium with fresh complete BrainPhys 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.

Summary

iCell GlutaNeurons can be thawed and cultured directly on MEAs where neuronal networks are established and the spontaneous electrical signals can be monitored. The methods presented here highlight the ease of using iCell GlutaNeurons 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.

Notes

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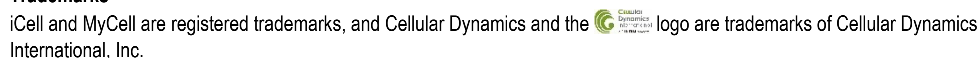

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