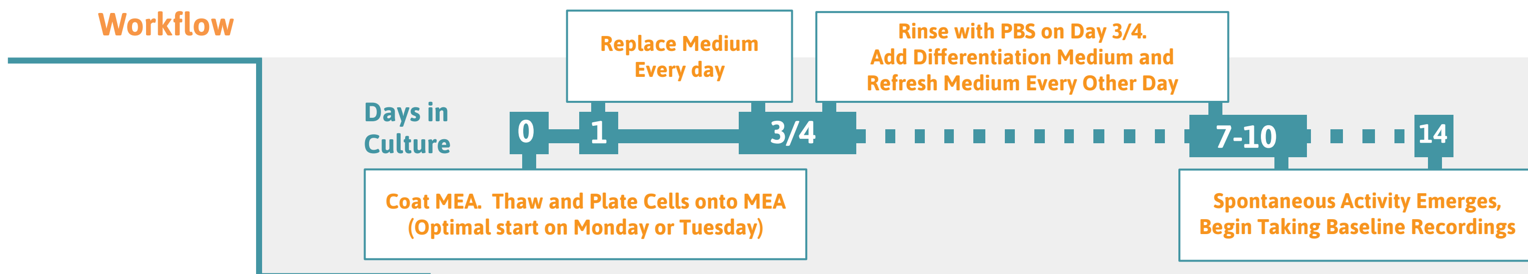
AXION BIOSYSTEMS

Application Protocol

SH-SY5Y Neuroblastoma Culture, Differentiation and Recording

Workflow



Preparing the MEA Plate

1. Add 5 µl of 0.01% poly-L-ornithine (PLO) solution to each well in the MEA plate.
2. Incubate the PLO-coated MEA plate in a cell culture incubator at 37°C, 5% CO_2 for 1 hour.
3. Rinse PLO from the culture surface with 200 µl of sterile DI water 4 times, then allow the MEA plate to air dry overnight.
4. Prepare 1mg/ml of laminin. Add 5 µl to each well in the MEA plate.
5. Incubate the laminin-coated MEA plate in a cell culture incubator at 37°C, 5% CO_2 for 2 hours.

Tip

Prepare the laminin fresh from frozen aliquots for every cell culture.

Tip

Recommended to add 6-8 mL of sterile water to the on-plate reservoirs to increase humidity.

Culturing and Plating SH-SY5Y Neuroblastoma

6. Prepare SH-SY5Y Neuroblastoma media using DMEM, supplemented with 10% FBS and 10 µM retinoic acid.
7. Dilute the SH-SY5Y Neuroblastoma cells (passage 3-5) with Neuroblastoma media to 1,600,000 cells/mL (*optimal cell density*).

   Note: Observations of cell concentrations at 800,000 cells/mL or less, show little to no activity, while concentrations at 3,200,000 cells/mL or greater, show reduced spike counts, so some optimization may be applicable.

8. Place a 5 µl droplet of SH-SY5Y Neuroblastoma cell suspension over the recording electrode area of each well of the MEA. See Figure 1 on page 2 for appropriate drop placement.
9. Incubate the MEA plate in a cell culture incubator at 37°C, 5% CO_2 for 3-4 hours.
10. Gently add 1/2 of the final volume of the medium to each well of the MEA. Adding the medium too quickly will dislodge the adhered neuroblastoma. Recommended final well volumes for each plate type are: 6- and 12-well = 1000 µl, 24-well = 500 µl, 48-well = 300 µl, 96 well = 200 µl.
11. Repeat step 10 a second time to reach the final recommended volume of medium.
12. Incubate in a cell culture incubator at 37°C, 5% CO_2.
13. For optimal cell health, replace media every day for 3-4 days.

Tip

Add a fresh preparation of retinoic acid during each feed to ensure its stability.

Tip

Ensure the neurons are evenly suspended before removing an aliquot to count.

Tip

Using a pipettor, add medium first in a semi-circle along the outer edge of the well. Progressively add medium to either side of the well so it fills evenly towards the center. The goal is to prevent a rush of medium in either direction that might dislodge the neurons.

Differentiation of SH-SY5Y Neuroblastoma into Neurons

14. Prepare Differentiation Media with serum-free DMEM supplemented with 1% Glutamax and 50 ng/ml BDNF.
15. After 3-4 days with 10 μM retinoic acid treatment, aspirate the media and rinse the cells gently with PBS.
16. Add Differentiation Media, and refresh every other day for 7-10 days.
17. Optimal recording time for spontaneous activity is between 10-14 days post differentiation. Activity will begin to decrease after 3 weeks. Change media at least 24 hours before recording activity

We would like to acknowledge and thank Oksana Pavlyuk Ph.D. of the Air Force Research Laboratory/RHMO at Wright-Patterson AFB in Dayton, Ohio, for developing this protocol and sharing the data associated with it.

Drop Placement

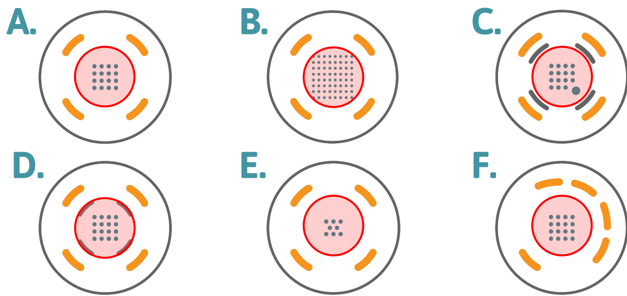


Figure 1: Drop Placement Diagram
The layouts above represent the bottom surfaces of wells in (A) a 48-well MEA, (B) a 6- or 12-well MEA, (C) a 24-well MEA or 48 well E-Stim+ MEA, (D) a 48-well AccuSpot MEA, (E) a 96-well MEA, and (F) a 48-well CytoView MEA. The number of electrodes per well is different across the plate formats, however the drop placement is the same, with the drop (red circle) centered on the recording electrodes and staying within the ground electrodes. On plate types with the addition of the stim-paddle in the lower right corner of the array, it is important to make sure the droplet covers this feature. The droplet may need to be manipulated after placement of the pre-treatment to ensure stim-paddle coverage.

Visualization of Typical Neuroblastoma Seeding Results

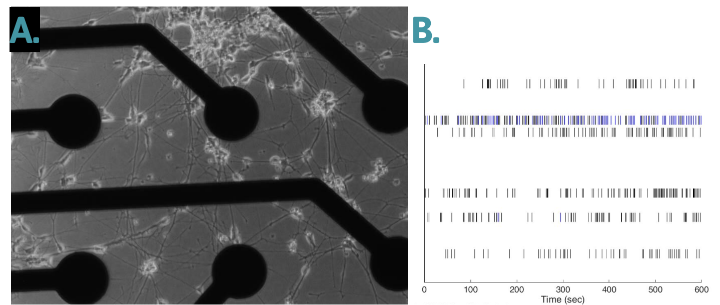


Figure 2: SH-SY5Y Post Differentiation Morphology and Activity
SH-SY5Y Neuroblastoma following 3 days of retinoic acid and 10 days of BDNF on a CytoView MEA, (A) 10x magnification. Notice the neurite formation and connectivity evident in the culture. (B) Representative raster plot of neural spike activity, visualized in the Neural Metric Tool. Black tick marks are individual spikes, while blue identify bursts.

Required Materials

Consumables

Item	Vendor	Catalog #
Axion MEA (6, 12, 24, 48, or 96-Well)	Axion BioSystems	
SH-SY5Y Neuroblastoma	Sigma	94030304-1VL
DMEM with Glutamax	Thermo Fisher	10569010
Brain-Derived Neurotrophic Factor	Alomone Labs	B-250
Retinoic Acid	Sigma-Aldrich	R2625
Fetal Bovine Serum (FBS)	Various	
0.01% Poly-L-Ornithine Solution	Sigma-Aldrich	P4957
Dulbecco's PBS without Ca2+/Mg 2+	Thermo Fisher	14040
Laminin		
100% Ethanol	Various	
15 mL and 50 mL Centrifuge Tubes	Various	

Equipment

Item	Vendor	Catalog #
Maestro Pro or Edge MEA System	Axion BioSystems	
AxIS Navigator	Axion BioSystems	NA
37°C Water Bath	Various	NA
Cell Culture Incubator	Various	NA
Hemocytometer or Cell Counter	Various	NA
Biological Safety Cabinet	Various	
Tabletop Centrifuge	Various	
Phase Contrast Microscope	Various	
Liquid Nitrogen Storage	Various	