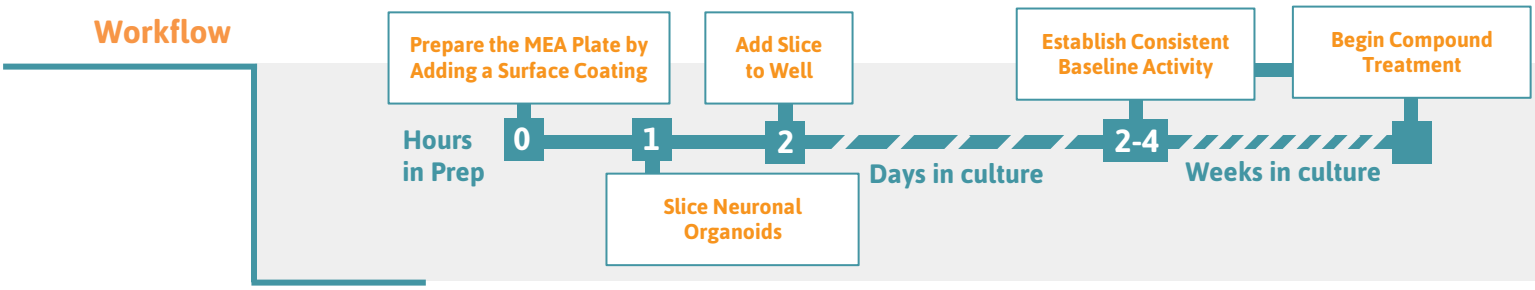
AXION BIOSYSTEMS

Organotypic Slice Protocol

from Stem Cell-derived Neuronal Organoids



Preparing the MEA Plate

1. Coat the entire well surface with a preferred substrate coating (e.g., poly-D-lysine, polyornithine, or 0.1% polyethylenimine).
2. Rinse the surface coating from the well with 200 µl of PBS 4 times, then allow the MEA plate to air dry for at least 2 hours.

Slicing Neuronal Organoids (>2 mm in diameter)

3. Transfer organoid(s) to a 35 x 10mm petri dish and remove as much of the solution as possible via pipetting and dabbing up with a kimwipe. Carefully pour chilled 4% agarose over organoids and allow to set. Use a metal spatula and pry the agarose block out of the petri dish. Using a razor blade, leave extra space around the organoids for specific trimming and mounting in the vibratome.
4. Mount trimmed block to mounting disk (supplied with vibratome) with super glue and allow the glue to set for at least 3 minutes.
5. Place mounting disk at vibratome and fill the vibratome chamber with ice-cold oxygenated (95% O_2 and 5% CO_2) ACSF solution.
6. Slice the neuronal organoids into ~250-400 µm slices. Use high blade frequency and a slow speed.

Positioning the Slice on the MEA Plate (Figure 1)

7. To transfer the slice to the well, use either a 1mL micropipettor or a transfer pipette. The tip of the pipette of choise should have wide enough bore size to aspirate the slice without damaging it, and this may require cutting the tip end off. The well to transfer to should contain enough warm ACSF solution volume to allow free movement of the slice into position on the electrode array.

Tip

Slowly heat 100 mL 1X PBS (beaker) on a heating stir plate set at 50°C and stir vigorously. Very slowly add 4 g of low-melting agarose powder to heated PBS and allow the solution to stir with heating till begins to thicken.

Tip

Orient the block such that the sample-containing end will encounter the blade first. If increased stability is necessary due to detachment during slicing, mount another block of 4% agarose behind the initial block containing the organoid(s).

Tip

Slices tend to reduce in thickness after some time in culture.

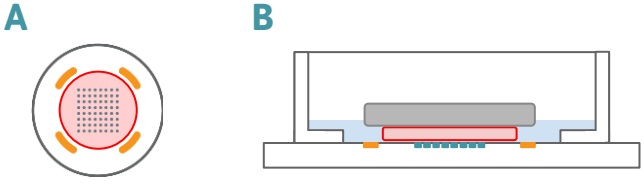


Figure 1: Slice Placement Diagram
The layouts above represent the bottom surfaces of wells in (A) a 6- or 12-well MEA. The number of electrodes per well between the plate formats and the slice placement is the same, with the slice (*red circle*) centered on the recording electrodes and staying within the ground electrodes. The side profile diagram in (B) demonstrates the slice anchor/grid (*grey*) holding the slice (*red*) down over the electrode array (*teal*), with ACSF (*blue*) just covering the slice to allow for sufficient oxygenation during culture and recording.

8. Using a fine-to-medium tip soft sample brush (e.g., histology camel hair brush) gently position the slice into the desired general orientation over the array and slowly remove the ACSF down to approximately 100-200 µL (enough volume to move the slice, but keep it contained over and touching the array).
9. Use the 1 mL micropipettor with an unmodified tip to gently remove the remaining ACSF, pushing the slice down onto the array via surface tension. It may be necessary to do this in stages – remove a little ACSF and then reposition the slice if it moves during solution removal. Repeat until ACSF is completely removed and slice is adhering to the array – there should be just enough solution (~100 µL) remaining to keep the tissue hydrated.
10. Leave the slice on the array for 20-30 seconds to adhere, repositioning as necessary.
11. Pre-wet the slice grid in ACSF and dab excess with Kimwipe. Place the slice grid on the tissue in one attempt if possible, trying diligently to avoid slice movement. It may be necessary to remove the grid, reposition the slice and try again, remaining conscious to avoid any additional slice movement.
12. Place ~50-200 µL of culture medium on the slice and grid, just enough to keep the tissue hydrated and healthy, and leave the slice in the well for 60 seconds to allow for functional adherence to the array.
13. Look at the final slice placement under the microscope to ensure the position is correct. If the slice is slightly off the target region, very carefully make final placement adjustments, but only if the movement is minute in scale.
14. Gently add 1/2 of the final volume of the medium to each well of the MEA and incubate in a cell culture incubator at 37°C, 5% CO_2 . Adding the medium too quickly will dislodge the grid. Recommended final well volumes for 6-well plate (1000 µl).
15. Next day, repeat step 8 to reach the final recommended volume of medium.
16. Exchange 50% of the medium every 2-3 days until the slice cultures are to be analyzed and/or proceed with preferred treatment. Stable baseline activity is usually observed 2-4 days after attachment to the electrodes, but depends on the maturation stage of the initial neuronal organoid (Figure 2).

Tip

The aCSF volume can be adjusted to allow for varying degrees of movement as required to position the slice.

Tip

Be patient with the slice anchor placement. Ideally, you only want to drop the anchor once. Small position adjustments may be possible with the anchor on top of the slice, but should generally be avoided. It will take some practice using the forceps and anchor to become accustomed to the challenging angles involved.

Tip

If significant repositioning is necessary, pull off the grid, add more ACSF to the well to detach the slice, and carefully reposition as needed.

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.

Neuronal Activity

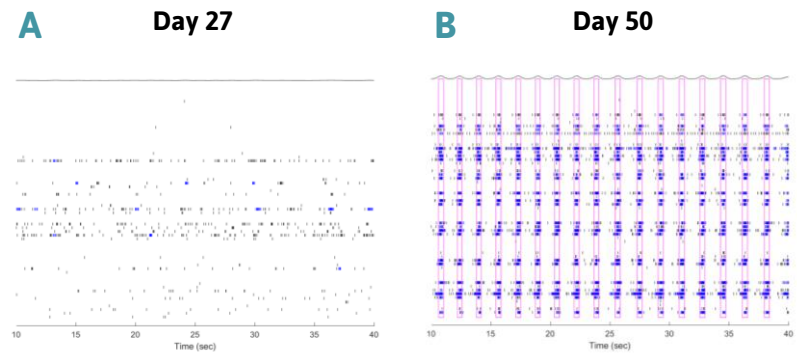


Figure 2: Neuronal Activity
Representative well-wide raster plots showing neuronal activity from 250 µm thick organotypic slices in the same well at 27 (A) and 50 (B) days post plating. Each tick indicates the time a spike occurred and each row indicates the electrode. Blue ticks indicate the spikes are of a single-electrode burst while black ticks are not. Ticks included in network bursts are outlined by magenta rectangles. The organotypic slices are spontaneously active by day 27, with a network burst phenotype emerging by day 50 in culture.
* Data courtesy by Dr Patapia Zafeiriou (UMG)

Required Material & Equipment

Item	Vendor
Maestro Pro or Edge MEA System, Axion MEA (6 or 12-Well)	Axion BioSystems
VT1200 Vibratome	Leica Biosystems
Slice Anchor/Grid (SHD-41/10, 64-1419)	Warner Instruments

Item	Vendor
Artificial Cerebrospinal Fluid (ACSF)	Various
Poly-D-Lysine (A3890401)	Thermo Fisher
50% Polyethylenimine Solution (P3143), Agarose (A9414)	Sigma-Aldrich