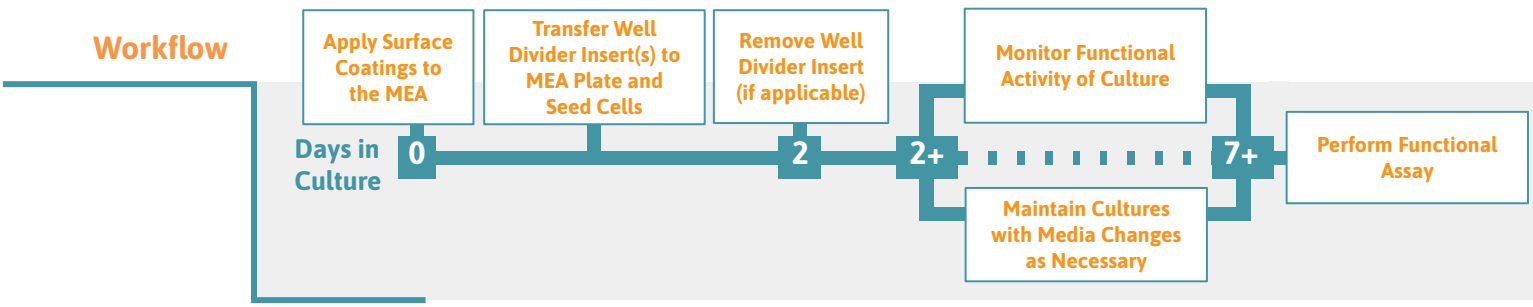
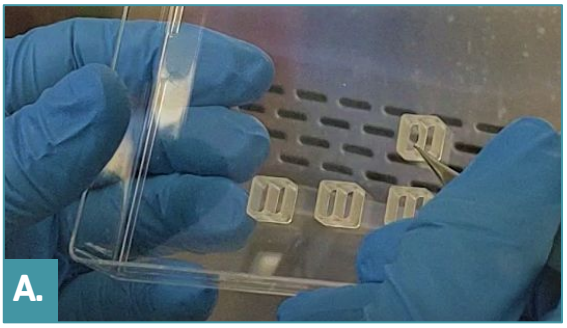
MEA Co-culture Protocol



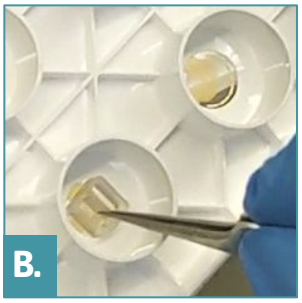
This protocol details the application and use of Well Divider Inserts (*Ibidi, cat. 80209*) on the CytoView MEA 6-well plate. The inserts specified in this document have two separate compartments that allow for compartmentalization of two cell populations on the array. Removal of the insert then allows the distinct cell populations to connect over time.



Prepare the MEA Plate

1. Spray the **CytoView MEA 6-well Plate Package** using 70% ethanol and transfer it to the biosafety cabinet.
2. Coat the wells of a CytoView MEA 6-well plate with the appropriate surface coating treatment (PEI/laminin, PDL, Fibronectin, etc.) and incubate for the time prescribed in your specific protocol.

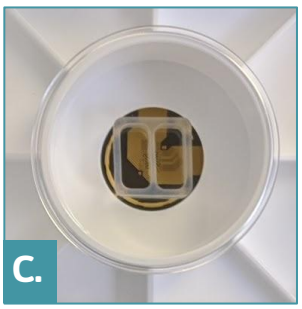
   Note: We recommend coating the entire surface of the well, as opposed to a droplet over the array, when using a **Well Divider Insert**.



Attaching the Well Divider Insert to the MEA plate

3. Spray the **Well Divider Insert Tray** (**Fig. 1A**) using 70% ethanol and transfer it to the biosafety cabinet.
4. Open the **Well Divider Insert Tray** and use a pair of tweezers to grasp a **Well Divider Insert** along the center divider (**Fig. 1B**). The bottom of the insert has an adhesive layer so removal from the container may require a gentle tug. Be sure to keep this adhesive layer face down when placing on the MEA surface.
5. Gently place the **Well Divider Insert** into one well of a 6-well plate such that the center line is over the middle of the microelectrode array (**Fig. 1C**). The insert divisions can be oriented either horizontally or vertically, by preference. Check the placement using a microscope, noting which electrodes are covered by the insert.

   Note: The **Well Divider Insert** will block approximately 2 columns or 2 rows (depending on orientation) of the microelectrode array.



6. If alignment is acceptable, use the blunt end of the tweezers to press down gently on the corners of the **Well Divider Insert**. This will secure the adhesive to the surface. Do not press on the divider in the center of the insert.

Figure 2: Functional Connectivity Between Distinct Cortical Networks
Rodent cortical neurons were plated in both compartments of a Well Divider Insert on a 6-well CytoView MEA plate. A) Each of the distinct cortical networks developed activity, synchrony, and oscillations consistent with standard cell culture protocols. B) The raster plot illustrates independent network activity by the two cortical networks, as well as functional connectivity between the two cortical networks.

Addition of the Cell Cultures

7. Prepare the cells for plating according to protocol requirements.
8. Add cells to each compartment of the **Well Divider Insert**. In each section of the insert, the cells may be added as a droplet (e.g. 5-10 µL) over the electrode array along the central divider, or by filling the entire area (e.g., >50 µL of cell suspension). See (**Fig. 3A-C**) for examples.
9. Transfer the plate to an incubator at 37 °C and 5% CO_2 to allow for cell attachment according to the standard cell culture protocol, typically 1 hour.
10. If the cells were plated in droplet form, add up to 100 µL per compartment to feed the culture and to prevent the droplet from drying out.

Insert Removal to Allow Functional Connection of the Co-cultures

11. Remove the **Well Divider Inserts** after the cells have attached to the surface (~1-2 days).
12. Using a pair of tweezers, grasp the center divider near the top or bottom edge of the **Well Divider Insert** and gently twist to lift.
    Note: If the insert is left in the well too long (e.g., 4+ days), the strength of the functional connection between the co-cultures may be impaired long-term.

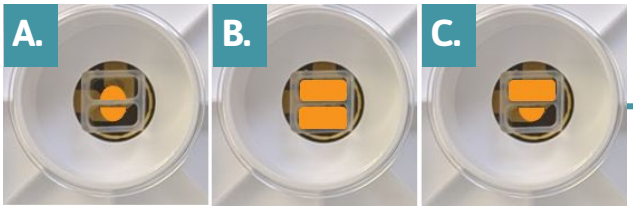


Figure 3: Potential Plating Scenarios for Compartments
The Well Divider Insert allows for cells to be plated in various scenarios such as: A) droplets to maximize the cells over the microelectrode array, B) whole compartment area for ease, or C) combinations of the two for various applications.

Required Materials

Item	Vendor
CytoView MEA 6 Plate	Axion BioSystems
Well Divider Insert (cat. no. 80209)	Ibidi
Surface Coating (Fibronectin, PEI, Laminin, etc)	Various
Cell Culture Media	Various

Item	Vendor
Maestro Pro or Edge	Axion BioSystems
AxIS Navigator	Axion BioSystems
Microscope	Various
Appropriate Micropipettes	Various
Tweezers	Various