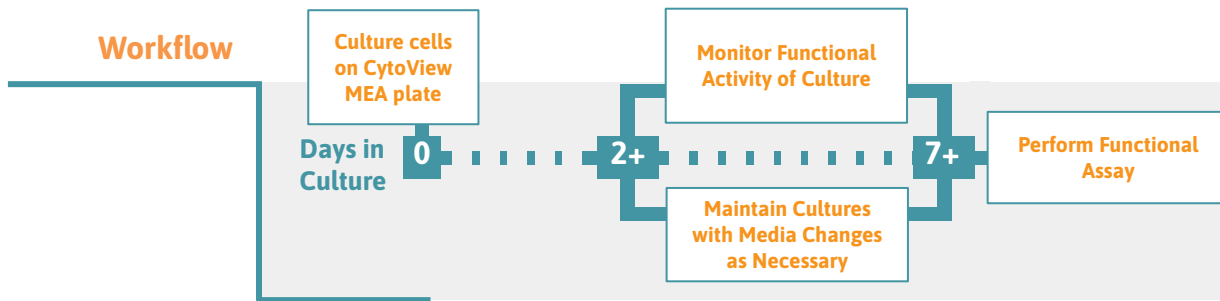
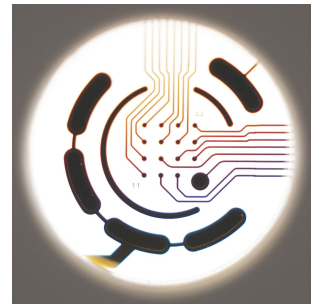


# MEA Viability Assay Protocol



This protocol details the use of impedance, via the **MEA Viability Module**, to monitor cell attachment on **CytoView MEA Plates**. Impedance measurements detect the presence of intact cell membranes on the same recording electrodes used to monitor functional activity of neurons and cardiomyocytes. As such, the **MEA Viability Module** can be used to track changes in cell coverage over time, implement quality control criteria, or evaluate the structural toxicity of drugs or chemicals. This protocol requires a **CytoView MEA Plate**, a **Maestro Pro** or **Edge**, and the **MEA Viability Module**.



## Cell Culture

1. Seed and culture the cells on a **CytoView MEA Plate** according to cell supplier recommendations.

**Note:** CytoView MEA plates are required for MEA Viability measurements.

## Perform a MEA Viability Measurement

2. Perform a MEA Viability measurement each time you measure the electrophysiological activity of the cells using a Viability configuration.
3. Place the **CytoView MEA Plate** in the **Maestro Pro** or **Edge**, and switch to the "Configuration" → "Neural + Viability Real-Time" → "Spontaneous + Viability" configuration or the "Configuration" → "Cardiac + Viability Real-Time" → "Field Potential + Viability" configuration.

4. Press **Record**. The MEA Viability measurement will be acquired first, followed by offset correction and the corresponding Neural or Cardiac measurement. Press **Record** again (or **Stop**) to end the measurement.

**Note:** The MEA Viability measurement takes 1 minute to complete. After the MEA Viability measurement is acquired, you may view the data in the **Activity Map** using the source selector drop-down menu.

5. Repeat steps 3-4 throughout the maturation of the culture, or, for a dosing experiment, before and after the perturbation.

**Note:** The absolute value of the MEA Viability measurement indicates cell coverage. However, it is best used as a relative measure to assess changes in cell viability.

### Tip

The viability data and electrical activity data are stored in the same RAW file for easy access and analysis with Axion software tools.

### Tip

Viability measurements are reported in units of "Resistance (kΩ)".

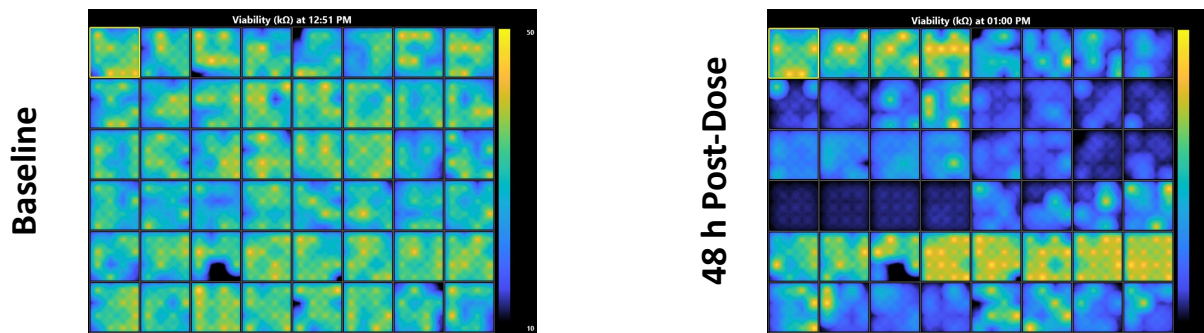
### Tip

Viability measurements are compatible with scheduled recording to collect measurements over time.

### Tip

The MEA Viability measurement includes the Resistance of the electrode itself (~8kΩ). For this reason, control compounds may be helpful to establish the range for covered vs. uncovered electrodes.





**Figure 1: Monitoring cell health with impedance following chronic dosing of hiPSC-derived neurons**

hiPSC-derived neurons (NeuCyte Labs) were seeded on a CytoView MEA 48-well plate and cultured for 14 days. The MEA Viability module was used to monitor cell attachment before (Baseline, left) and after (Post-Dose, right) the addition of neurotoxic compounds. The impedance measurements were acquired at 0.5, 4, 24, 48, and 72 hours post-dose.

## Analyze Viability Data in AxIS Navigator

- Load a **RAW** file into **AxIS Navigator** using the "File → Open Recording" menu, or by dragging the **RAW** file into the stream tree.
- Right click to apply the appropriate configuration (e.g. **Spontaneous + Viability**).
- Press **Record** to begin the analysis, producing a **Statistics Compiler CSV** file.
- Repeat steps 6-8 for each recording you intend to analyze.

**Note:** Batch processing may be used to process multiple MEA Viability files with the same configuration. Additional analysis may be performed with the Neural Metric Tool (NMT) or Cardiac Analysis Tool (CAT).

## Create Viability Data Figures with the AxIS Metric Plotting Tool

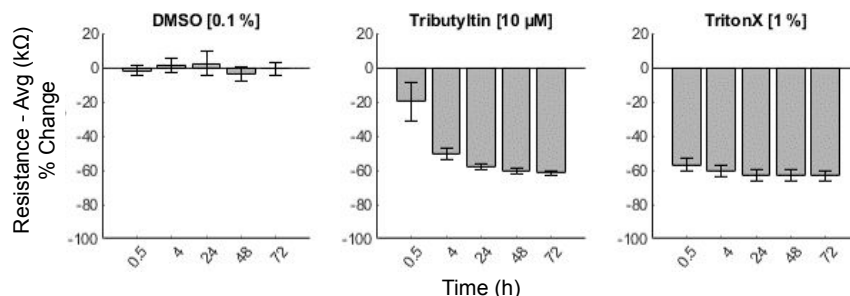
- Press the "Baseline" button in the **AxIS Metric Plotting Tool** to load a **Statistics Compiler CSV** file.
- Press the "Comparison" button to select any additional **Statistics Compiler CSV** files for analysis.
- Select "Resistance - Avg (kΩ)" from the Metric drop-down menu.

**Note:** Using a control compound, like Triton X-100, that lyses the cell membrane can be helpful when analyzing MEA Viability measurements in a dosing experiment. For further details, see the MEA Viability FAQ in the AxIS Navigator User Guide.

- Repeat steps 10-12 for each experiment.

## Required Materials

Item	Vendor
CytoView MEA Plate	Axion BioSystems
Maestro Pro or Edge	Axion BioSystems
AxIS Navigator (version 3.4 or higher)	Axion BioSystems
MEA Viability Module	Axion BioSystems
AxIS Metric Plotting Tool (version 2.4.4 or higher)	Axion BioSystems
Surface Coating (Fibronectin, PEI, Laminin, etc)	Various
Cell Culture Media	Various



**Figure 2: Neurotoxicity assay with hiPSC-derived neurons**

The Vehicle control (0.1% DMSO) had no effect on viability as quantified through resistance measurements. Triton X-100 (1%) lysed the cell membranes, serving as a positive control. Tributyltin (10  $\mu$ M) gradually killed the hiPSC-derived neurons over the first 24 hours.

