MED64 PRESTO

Discover the beauty that only the highest-sensitivity MEA can bring to your research!

MED64 Protocol

Axol Human iPSC-derived Sensory Neuron Progenitor

axol

ALPHA MED SCIENTIFIC

Version: 1.10; May 1, 2018

Alpha MED Scientific Inc.
Saito Bio-Incubator 209, 7-7-15, Saito-asagi,
Ibaraki, Osaka 567-0085, Japan
E-mail: support@med64.com
Website: http://www.med64.com

Contents

1. Introduction

Stem cell derived neurons provide insights into the mechanisms of the nervous system, behavior, and disease. Sensory neurons that transduce pain are crucial to protect an organism from potentially damaging external stimuli. Dorsal root ganglions (DRG) are pain-related neurons that have a variety of sensory receptors that are activated by chemical, thermal, and mechanical stimulation. Due to the complex yet subtle activity of stem cell derived sensory neurons, a consistent and reliable assay is needed to measure and examine their activity. The MED64 Presto is a high fidelity microelectrode array (MEA) platform that is engineered to detect a broad range of action potentials that stem cell derived sensory neurons produce.

The MED64 Presto's broad acquisition bandwidth combined with superior signal to noise ratio and carbon nanotube technology affords unblemished extraction of basic electrophysiological variables. The combination of precise extraction of electrophysiological activity with the ease of culturing cells directly onto the planar microelectrodes makes the MED64 Presto ideal for pharmacological, drug safety screening, and basic scientific applications. The planar micro-electrodes on the MED64 MEA Plate have the lowest impedance of any micro-electrode array, making the MED64 Presto ideal for acquiring data from spontaneously spiking neurons or neurons that fire in response to drug application. The MED64 Presto also has high capacitance electrodes, enabling the MED64 to deliver high stimulating current, which is essential for evoked response studies.

The goal of this protocol is to describe how to set up experiments with dissociated sensory neuron cultures, acquire relevant data, and extract the data for presentation or publication. This material has been prepared by scientists with expertise in neuroscience and electrophysiology. A complete protocol for plating and culturing neuron cultures has been prepared based on user experience.

1-1. Acknowledgement

Alpha MED Scientific would like to thank the MED64 users that have shared their knowledge,:

Ikuro Suzuki,PhD - Tohoku Institute of Technology
Aoi Odawara,PhD - Tohoku Institute of Technology
Zoe Nielsen,PhD - Product manager, Axol Bioxcience
Michael Trujillo,PhD - Global product manager, Alpha MED Scientific

1-2. Disclaimer

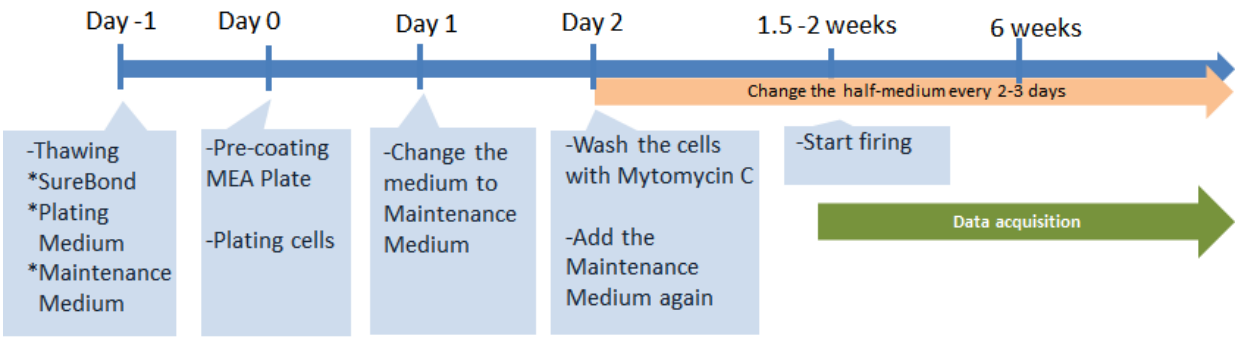
This application note is a summary of information shared by MED64 users and is to be considered marketing material. These methods have been developed, tested, and verified over the course of projects published in peer-reviewed literature. However, Alpha MED Scientific does not guarantee that the information written in this document is correct and is free from all liabilities. Please refer to the scientific literature for further insight on these techniques, as well as the MED64 Presto and MEA Symphony software manuals for detailed instructions on use of the MED64 Presto System.

2. Pretreatment of the MEA Plate and plating sensory neurons

2-1. Material to be prepared

Items	Supplifer	Cat. No	Note
Cell			
Human iPSC-Derived Sensory Neuron Progenitor	Axol	ax0055	>500,000 cells / vial
Reagents for coating			
SureBond+ReadySet	Axol	ax0041	
(SureBond)			Store at -80 °C
(ReadySet)			Store at 4 °C
Reagents for cell plating and cultureing			
Neural Plating-XF medium	Axol	ax0033	Store at -80°C
Sensory Neuron Maintentance Medium	Axol	ax0060	Store at -80°C
Mitomycin C	Sigma	M4287	Store at -20°C
Glial-Derived Neurotrophic Factor (GDNF)	Peprotech	450-10	Store at -20°C
Nerve Growth Factor (NGF)	Peprotech	450-01	Store at -20°C
Brain-Derived Neurotrophic Factor (BDNF)	Peprotech	450-02	Store at -20°C
Neurotrophin-3 (NT-3)	Peprotech	450-03	Store at -20°C
Equipment			
MEA 24well Plate-comfort	Alpha MED Scientific	MED-Q2430L	
CellSpotter-24comfort	Alpha MED Scientific	MED-CRS24L	
Cloning ring	Iwaki	11-0162 (RING-05)	ID:3.4, OD:5, Height:10 (mm)
35mm culture dish	Various		
Conical tube (15, 50 ml)	Various		
Microtube (2.5ml)	Various		
Pipet, Petri dishes, kim wipes, waterbath, clean bench, CO2 incubator, Centrifuge, microscope			

2-2. Workflow



2-3. Preparation of Regents

- Please read product manual and protocol by Axol or other suppliers.

Neural Plating-XF Medium

- Upon receipt, store Neural Plating–XF Medium (Axol Cat# ax0033) at or below -80°C and protected from light.
- When ready to use, thaw Neural Plating–XF Medium overnight at 4°C in the dark.
- Once thawed, Neural Plating–XF Medium must be used and cannot be refrozen.

Sensory Neuron Maintenance Medium

- Upon receipt, aliquot and store Sensory Neuron Maintenance Medium (Axol Cat# ax0060) at or below -80°C protected from light.
- When ready to use, thaw an aliquot of Sensory Neuron Maintenance Medium overnight at 4°C in the dark. (Thawing the Sensory Neuron Maintenance Medium can take longer than overnight.)
- Prepare Sensory Neuron Maintenance Medium by adding the following:

Growth Factor	Final Concentration
Glial-Derived Neurotrophic Factor (GNDF)	25 ng/mL
Nerve Growth Factor (NGF)	25 ng/mL
Brain-Derived Neurotrophic Factor (BDNF)	10 ng/mL
Neurotrophin-3 (NT-3)	10 ng/mL

- Growth factors should be added fresh each time an aliquot of Sensory Neuron Maintenance Medium is thawed.
- A thawed and supplemented aliquot of Sensory Neuron Maintenance Medium can be stored at 4°C for 1 week.

Mitomycin C

- Prepare a 0.5 mg/mL stock concentration of mitomycin C by solubilizing 2 mg in 4 mL of ddH2O. Make 50-100 µL aliquots of mitomycin C (0.5 mg/mL), protect from light and store in a dark box at 4°C. Stored at 4°C, mitomycin C is stable for up to 8 weeks.

Sensory Neuron Maintenance Medium containing Mitomycin C

- Prepare medium containing 2.5 µg/mL of mitomycin C by adding 100 µL of the 0.5 mg/mL stock of mitomycin C to 20 mL of Sensory Neuron Maintenance Medium.
- This medium should then be filter sterilized prior to use using a 0.22 µM filter.

2-4. Pretreatment of the MEA Plate

CAUTION:

- Avoid contact with the electrodes in all of following procedures as they are extremely fragile.

Pretreatment of the MEA plate is the most critical step to succesfully culture neurons onto the electrodes of the Presto MEA Plate. The surface of a new Presto MEA Plate is hydrophobic. Therefore, hydrophilization of the Presto MEA plate is necessary to enhance the adhesion of neurons to the electrodes of each well. If cells do not adhere to the electrodes, you will not be able to record from them. It should be noted that some coating agents can affect neural activity, viability, degree of neurite outgrowth, extent of migration, and longevity. Improper coating techniques can cause large-scale clumping and/or the death of neurons even if all other cell culture steps are performed properly. The following section contains recommendations for appropriate pretreatment of the MEA Plate.

Sterilizing the MEA Plate

1. Rinse a new MEA Plate with sterilized distilled water (SDW) at least three times. Clean it with 70% ethanol several times (or immerse it in 70% ethanol for 15 minute).
    - It is strongly recommended to immerse the MEA Plate in 70% ethanol for 15 minutes when a Presto MEA Plate is reused.
    - Higher-grade ethanol is recommended to avoid deposits of organic substances onto the MEA Plate after drying.
2. Rinse the Presto MEA Plate with sterilized distilled water (SDW) at least three times.
3. Let the Presto MEA Plate dish dry under ultraviolet light to irradiate for 15-30 min. Store and handle the Presto MEA Plate in a sterilized container.

Pre-coating the MEA Plate

- Thaw the Surebond (Axol Cat# ax0052) at 4 °C overnight the night before plating.

1. Add 450 μL of Readyset (250 uL / Cm2) into the MEA 24well Plate-comfort, and incubate at 37ºC for 45 minutes.
2. Remove the Readyset solution, and immediately rinse the MEA Plate with sterilize water 4 times. Fill the wells in the MEA Plate with sterilize water.
    - Pay attention so that the plate surface dry during this process.
3. Mix SureBond 120uL with D-PBS 6ml and pipette it.
4. Remove sterilized water from the MEA Plate and immediately fill the Probe with 380 uL of SureBond solution (#3).
5. Incubate in an incubator (37 °C, 5%CO_2) for one hour.

2-5. Thawing and Preparing the sensory neurons

- Please read product manual and protocol by Axol or other suppliers.

1. Transfer the vial of Axol sensory neurons (Axol Cat# ax0055) from storage by transporting the vial submerged in dry ice. Remove the vial from dry ice and transfer it to a 37ºC water bath.
2. Quickly thaw the vial of sensory neurons in a 37 °C water bath. Do not completely submerge the vial (only up to 2/3rd of the vial). Remove the vial before the last bit of ice has melted, after 1-2 minutes.
    - Do NOT shake the vial during thawing.
3. Take the vial of sensory cells to a biological cabinet, spraying the vial and hood thoroughly with 70% ethanol and wiping with an autoclaved paper towel before placing the vial in the hood.
4. Using a p1000 pipette, transfer the cell suspension into a 15mL sterile conical tube. Gently wash the cryogenic vial with 1mL of warm Neural Plating-XF Medium and transfer this to the 15mL sterile conical tube.
5. Add 8 mL of Neural Plating-XF medium drop-wise to the cell suspension.
6. Centrifuge cells at 200 xg for 5 minutes at room temperature.
7. Aspirate and discard the supernatant carefully with a pipette.
8. Using a P1000 pipette, gently resuspend the cell pellet in 1 mL of Neural Plating-XF Medium (ax0033) until they are in a single cell suspension.
9. The ideal cell volume is 1,000,000 cell/ml.

2-6.Plating the sensory neurons onto the MEA Plate

CAUTION:

Be careful NOT to touch the electrodes.

Preparing the CellSpotter

The sensory neurons will be plated onto electrodes in a Presto MEA Plate using the CellSpotter-comfort24 + cloning rings. Prepare the CellSpotter as well as cloning rings before plating.

Figure 1. CellSpotter-comfort24(left) and cloning rings (right). Cells will be amounted onto the recording electrodes through cloning rings placed in the center-hole of the CellSpotter.

1. Autoclave the CellSpotter and cloning rings.
2. Place the cloning rings in a petri dish (Figure 2, Left).
3. Shake the petri dish. Select only cloning rings that do NOT move (Figure 2, Right).
    - Some cloning rings have an uneven edge, which can cause cell suspension to leak through the bottom. This step is necessary to select only the good cloning rings.

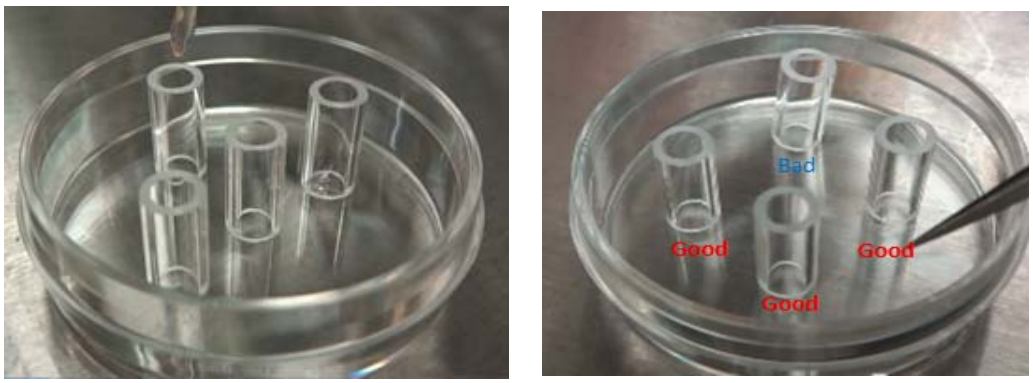


Figure2. Cloning ring placed in a petri dish (left). Shake it and select only cloning rings that do NOT move (right).

4. Moisten the inside of the cloning ring by pouring Neural Plating-XF Medium (Figure 3, left).
    - This process will help prevent the cell suspension from sticking in the middle of cloning ring (Figure 3, right).

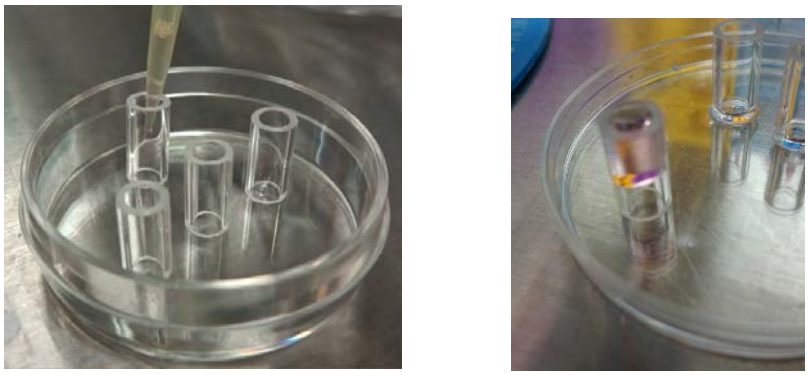


Figure 3. Moisten the inside of the cloning rings (left) and cell suspension sticking in the middle of cloning ring (right).

Plating cells onto electrodes using the CellSpotter

1. Mount the CellSpotter onto the MEA plate (Figure 4, left)
2. Remove the SureBond from each well in the Presto MEA plate through the side-hole (Figure 4, right).

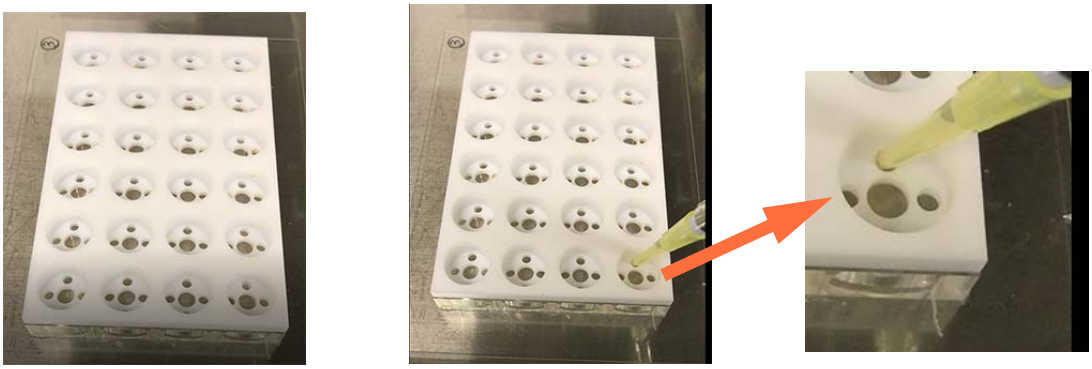


Figure 4. Placing the CellSpotter onto the MEA plate (left) and remove the SureBond from a well through the side-hole (middle, right).

3. Insert the cloning rings into the center-holes of the CellSpotter.

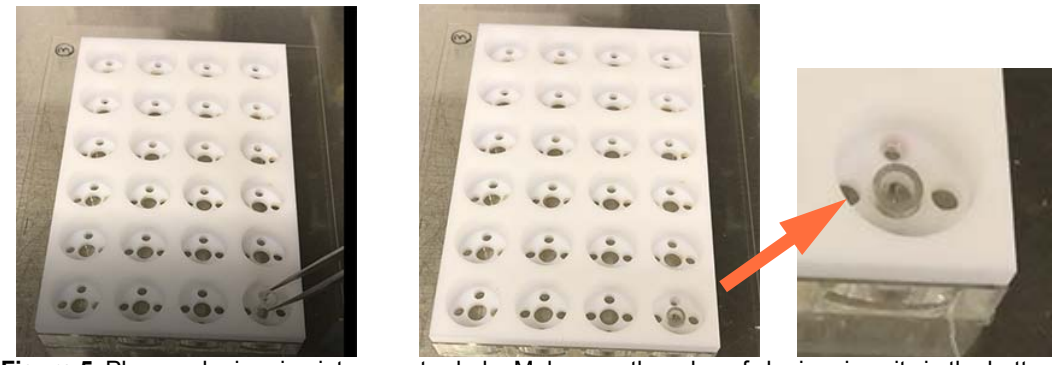


Figure 5. Place a cloning ring into a center-hole. Make sure the edge of cloning ring sits in the bottom of the MEA Plate.

4. Flood around 75 uL cell suspension (containing around **75,000 cells**) into the cloning ring.

   - **Final cell density: 8.0 x 10^5 Cm^2**

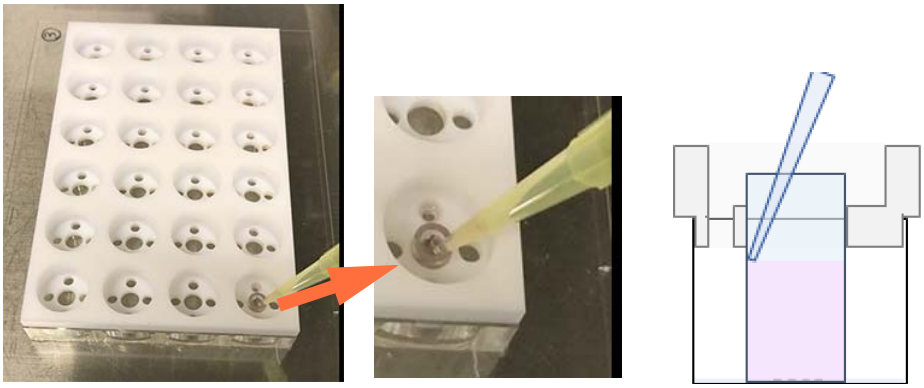


Figure 6. Flood the cell suspension into the cloning ring placed in the center-hole.

5. Add 500 µL of Neural Plating-XF Medium to the wells through the side-holes (Figure 7).

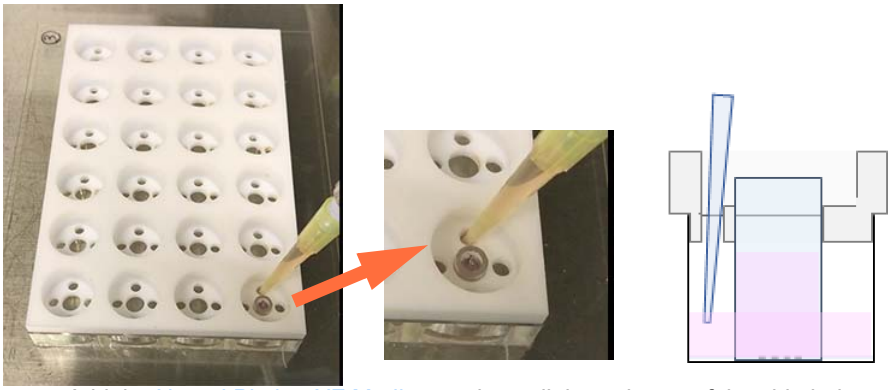


Figure 7. Add the Neural Plating-XF Medium to the well through one of the side holes.

6. Plate the neurons onto electrodes in the all wells using the same procedure (#2-5).

7. Incubate at 37ºC, 5%Co_2 for one hour.

8. Remove gently the cloning rings for all wells (Figure 8, left) and then CellSpotter (Figure 8, right).

Figure 8. Remove the cloning rings (left), and then CellSpotter after removing all coning rings.

9. Add 500 µL of Neural Plating-XF Medium to each well.

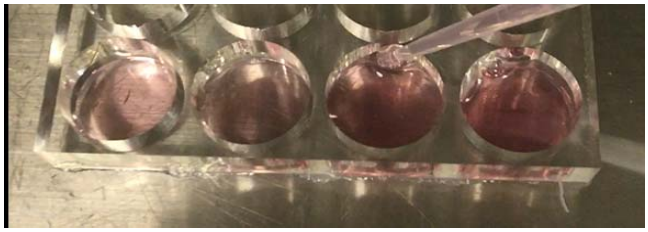


Figure 9. Add 500 µL of Neural Plating-XF Medium to each well.

On the following day (Day 1)

10. Replace the medium with 500 µL of fresh pre-warmed, 37ºC Sensory Neuron Maintenance Medium (ax0060) Supplemented with GDNF (25 ng/ml), NGF(25 ng/ml), BDNF(10 ng/mL), NT-3(10 ng/mL).

On the second day after thawing (Day2)

11. Remove all the culture medium in each well of the MEA plate, and replace with 500 µL of Sensory Neuron Maintenance Medium containing 2.5 ug/mL of mitomycin C.

12. Incubate the Presto MEA plate for 2 hours at 37ºC, 5%CO_2.

13. Remove the Sensory Neuron Maintenance Medium containing 2.5 ug/mL of mitomycin C from the wells in the Presto MEA Plate, and gently wash the cells in the MEA plate once with pre-warmed 37ºC D-PBS (1x) (without calcium or magnesium).

14. Fill each well with 1 mL of 37ºC Sensory Neuron Maintenance Medium supplemented with GDNF (25 ng/mL), NGF (25 ng/mL), BDNF (10 ng/mL).

    - Mitomycin C treatment is not effective immediately. Non-neuronal cell death will not occur until 4-5 days after treatment. Full effects will be apparent after 7 days.

15. Replace half the medium volume with fresh pre-warmed 37ºC Sensory Neuron Maintenance Medium supplemented with GDNF(25 ng/mL), NGF(25 ng/mL), BDNF (10 ng/mL), NT-3 (10 ng/ml) every 3-4 days.

16. Sensory neuron starts firing 1-2 weeks after plating, however you may want to perform your experiments 6 weeks or later after synchronized firing is observed.

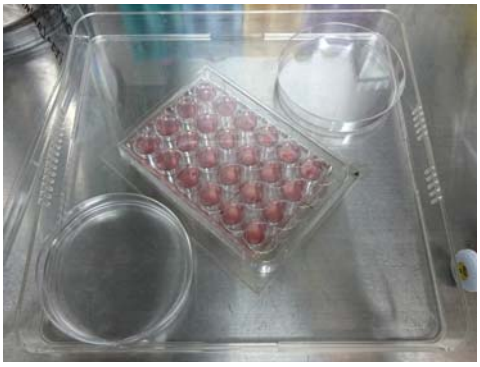


Figure 10. Cultured sensory neurons plated in an MEA Plate.

2-6. Cleaning the used Presto MEA Plate

The MED64 Presto MEA Plate's electrical characteristics are best during the first use. High quality signals can be recorded and effective stimulation is possible with the Presto MEA Plate thanks to the electrode's characteristics, the lowest impedance in a commercially available microelectrode array. The electrode's impedance will increase with repeated use of the MEA Plate due to damage in handling and/or residual cellular debris and coating materials. However, the MEA plates can be re-used if they are handled and cleaned very carefully. The following are cleaning procedures recommended by MED64 users:

- **CAUTION:**

  ALWAYS avoid contact with the surface of the MEA Plate to preserve the microelectrodes and insulation layer.

1. Clean the MEA Plate with distilled water.
2. Fill all wells in the MEA Plate with 0.25% Trypsin, and incubate at 37°C for 1 hour.
3. Rinse the MEA Plate with distilled water.
4. Clean the MEA plate in a ultrasonic bath for 3 minutes.
5. Immerse the MEA plate in 70% ethanol for 15 minutes.
6. Store the MEA plate in a distilled water.

When re-used:

1. Immerse the MEA Plate in 70% ethanol for 15 minutes.
2. Rinse the MEA Plate with distilled water.
3. Let it dry under ultraviolet irradiation (Irradiate both sides for 30 min each).
4. Proceed to pre-coating as soon as possible.

3. Data acquisition

Cultured sensory neurons start firing 1-2 weeks after plating, but initial activity can vary depending on culturing technique and conditions. Once the neurons begin firing spontaneously, activity can be recorded using the MED64 Presto, however you may want to perform your experiments 6 weeks or later after synchronized firing is observed. The following section details the recommended environment for recording from sensory neurons.

3-1. Recommended experimental environment

37°C is the typical recording temperature recommended for recording from sensory neuron cultures. However, the set temperature can vary depending on samples and nature of your experiments.

The MED64 Presto Amplifier incorporates heater, that heats the MEA Plate from the bottom. Below are the recommended settings for data acquisition.

- Do NOT place the MED64 Presto Amplifier in an environment where temperature changes frequently, for example in the proximity of an air conditioner or heater.
- Keep the acrylic lid closed and secure during acquisition.
- It can take time for the set-temperature to stabilize. Power on the temperature controller at least 30 minutes before starting acquisition.

If/When the set-temperature is changed, wait for the temperature reported by the temperature controller to stabilize at the new temperature. It can take 5-30 minutes or more but is typically very fast.

3-2. Data acquisition and analysis

Each well in the MEA 24-well plate has 16 recording electrodes as well as 16 reference electrodes. The differences between the field potential acquired at each recording electrode is compared to the potential of the average for all reference electrodes. Acquired signals are amplified by x1000, digitized, and sent to an acquisition PC.

MEA Symphony software is available for data acquisition and analysis. The Symphony software always acquires extracellular signals with acquisition bandwidth of 0.1Hz - 5 kHz. However, acquired raw data can be filtered for analysis. The following parameters are typically recommended for analyzing spikes recorded from sensory neurons.

- Low-pass filter (2 pole): Bessel 3000Hz
- High-pass filter (2 pole): Butterworth 100Hz (Select 1Hz if you want to record slow wave)

3-3. MEA Symphony software

The MEA Symphony is an acquisition and analysis software for the MED64 Presto System.The MEA Symphony records raw extracellular signals at all 384 electrodes, extracts spikes, and analyzes the spike frequencies. The software also extracts synchronized bursts over every electrode in a well and analyze those synchronized bursts.

The MEA Symphony always records and **saves raw data (**0.1Hz - 5kHz acquisition bandwidth) no matter what filtering option is selected. However, filtered data is used for all display and analysis.

This section will quickly guide you capabilities of the Symphony software.

Figure 11. Main screen for the MEA Symphony software.

Oscilloscope

There are several ways to displayed acquired data such as:

- Raw data acquired from all 384 electrodes
- Raw data acquired from all electrodes in a selected well
- Raw data acquired from an electrode in a selected well (with spike extractions)
- Extracted spikes from all electrodes in a selected well
- Heat map for a selected well

Analysis

The MEA Symphony provides abundant of spike and burst analysis. All analysis are displayed as a graph in the Main screen, and can be included in your Analysis report as well as exported independently to CSV file.

Spike frequency analysis

The following spike frequency analyses are available:

- The total number of spikes (Per electrode, Per well, Per treatment).
- Spike frequency (Per electrode, Per well, Per treatment).
- Inter-spike interval (Per electrode, Per well, Per treatment).
- Time course of spike frequencies (per well).Number of spikes (Per electrode, Per well, Per treatment).

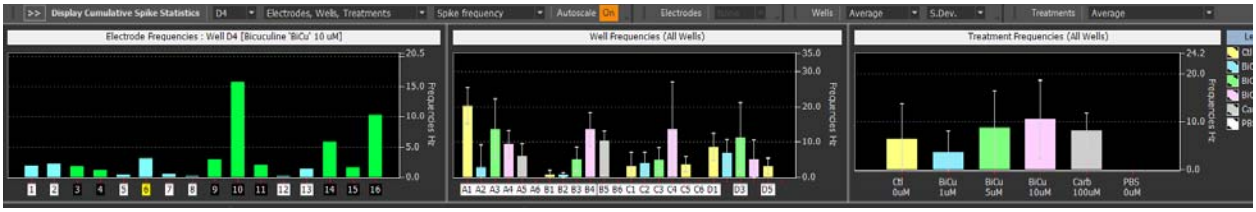


Figure12. Spike frequency analysis performed with the MEA symphony per electrode (left), well (middle), and treatment (right).

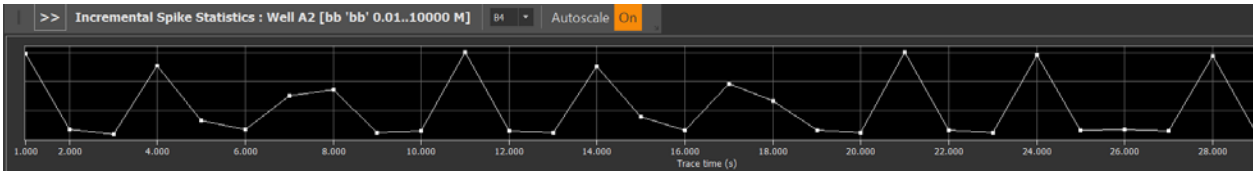


Figure 13. Time course of spike frequency analysis (per a well) performed with the MEA Symphony.

Burst analysis

Following analysis is available for synchronized bursts (bursts synchronized over electrodes in a well):

- Raster plots (with burst extractions) for a selected well
- Spike frequency histogram
- Burst analysis information:
  Number of bursts, Start time, End time, Burst duration, Inter-burst-intervals, Number of spikes in a burst (per a well)

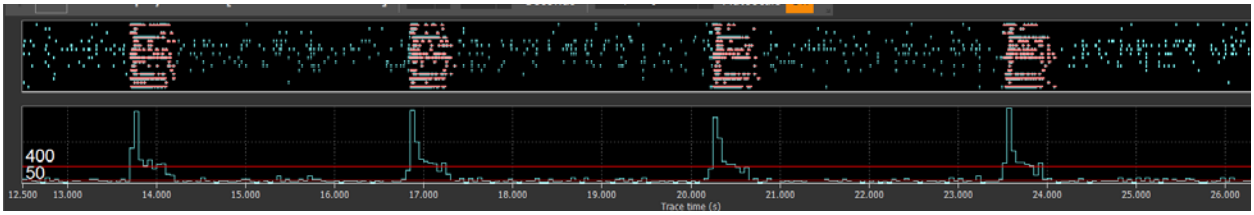


Figure 14. Raster plots (top) and Spike frequency histogram computed (bottom) and displayed in the MEA Symphony.

Exporting

Following data or/and analysis can be exported with the MEA Symphony.

	Format	
Raw data	CSV Text (Ascii)	
	Binary short 16-bit	
	Mobius (.modat)	
Spike	CSV Text (Ascii)	Time stamp only
	CSV Text (Ascii)	Time stamp and spike waveforms
Time course of Spike Frequencies	CSV Text (Ascii)	
Spike analysis per electrode	CSV Text (Ascii)	Number of spikes, Spike frequencies, Spike intervals
Spike analysis per well	CSV Text (Ascii)	Number of spikes, Spike frequencies, Spike intervals
Spike analysis per treatment	CSV Text (Ascii)	Number of spikes, Spike frequencies, Spike intervals
Burst analysis	CSV Text (Ascii)	Number of bursts, Start time, End time, Burst duration, Inter-burst-intervals, Number of spikes in a burst (per a well)

May 1, 2018

Alpha MED Scientific Inc.
Saito Bio-Incubator 209, 7-7-15, Saito-asagi,
Ibaraki, Osaka 567-0085, Japan
Phone: +81-72-648-7973 FAX:+81-72-648-7974
http://www.med64.com support@med64.com

Manufactured by **Alpha MED Scientific Inc.**