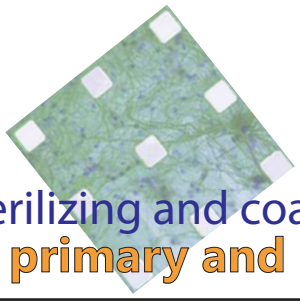


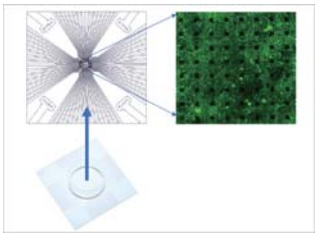
MED64

Sterilizing and coating MED Probes before plating primary and iPSC-derived cell cultures

Introduction

Caution: Do not touch electrodes located in the center of the MED Probes

MED Probes have microelectrodes composed of platinum black or carbon nanotube, which are insulated with polyimide (or acrylic), all of which are hydrophobic materials. To make the surface is more hydrophilic, the MED Probe must be coated so that cells will grow over the surface of the electrodes. For optimal recordings, cells must be plated over the central electrode grid but not on the reference electrodes near the edges of the probe.



Materials

1) Ethanol
2) Distilled water
3) UV light source
4) PEI (Optional)
5) Laminin-511 and poly-D-lysine (neurons)
6) Matrigel® or fibronectin (cardiomyocytes)

Sterilization

Do not autoclave

1) Rinse probes with 70% ethanol several times or immerse for < 15 mins.
2) Aspirate ethanol (under sterile hood) and rinse at least 4x with SDW.
3) Aspirate remaining distilled water and briefly dry under UV illumination.

Primary and iPSC-derived neuron cultures

We recommend either of following method to coat MED Probes before plating neuronal cultures.

Method 1:

1) Rinse MED Probe 3x with SDW.
2) Add 1mL of Poly D Lysine (50 ug/ml) to the center of array and incubate at 37 ℃ for 1 h.
3) Remove the PDL from the MED Probe. Rinse the MED Probe 4x with SDW.
4) Dry the MED Probe on a clean bench (for around 1h).
5) Add 1ml of Laminin 511 (2 ug/ml) and incubate at 37 ℃ for 1h.
6) Aspirate the Laminin 511. Plate cells ASAP.

* Remove the bubbles on the electrodes if there are any.
* Plate cells BEFORE electrodes are dried.

Method 2 :

1) Treat the surface of MED Probe with 0.005% Polyethyleneimine (PEI) in 25 mM borate buffer (pH 8.4) for 10 minutes at room temperature (See the next page for details).

* You may need to change the concentration and the time for PEI treatment depending on your cells and experiments.

2) Remove the PEI and rinse the MED Probe 4x with SDW.
3) Dry the MED probe on a clean bench overnight.
4) Add 1mL of Lamin 511 (2 ug/ml) and incubate at 37 degree for 1hour.
5) Aspirate the lamin 511. Plate cells ASAP.

Cardiomyocytes

We recommend using Matrigel® or fibronectin to coat MED Probes before plating cardiomyocyte cultures.
1) Obtain sterile MED Probe, pipette 7 µl Matrigel® (200 µg/ml) or 2 µl fibronectin (50 µg/ml) into the center of the recording electrode array and incubate at 37°C for 1 h (Matrigel®) or at 4°C overnight (fibronectin).
2) Aspirate coating solution and plate cells (total volume 2-7 µl) in the middle of the array by pipetting a small drop (or by using a cloning cylinder). Incubate 2-5 h, allowing cells to adhere.

* You may need pre-coating with PEI depending on your type of cells and experiments. See the next page for the PEI coating.

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* Caution: Do not touch electrodes located in the center of the MED Probes

MED Probe (MEA) optional PEI-coating instructions for cell culture preparations

Materials

Caution: Do not touch electrodes located in the center of the MED Probes

1) 25 mM borate buffer - sodium tetraborate (Sigma S9640) + HCl (to adjust pH)
2) 50% (w/v) Polyethyleneimine (PEI) in water (Sigma P3143)
3) Parafilm wax
4) Distilled water

PEI Coating Protocol

1) Make 25 mM borate buffer, as follows:
    - Dissolve 4.768 g $Na_2B_4O_7 \cdot 10\ H_2O$ in 450 ml sterile distilled water
    - Adjust pH to 8.4 with HCl
    - Add distilled water to final volume of 500 ml
2) Dilute PEI to a 1% stock solution in water
3) Dilute PEI again to 0.005% min borate buffer
4) Pipette 0.7 ml of PEI in borate buffer onto each MED Probe (enough to cover)
5) Seal MED Probes with parafilm wax (to reduce evaporation) and coat for 10 mins at room temerature.

• You may need to change the concentration and time for the PEI treatment depending on your cells and experiments.

6) Aspirate PEI solution (avoid touching electrodes) and rinse 4x (or more) with sterile distilled water.
7) Store PEI-coated MED Probes under 1 ml distilled water, sealed with parafilm wax at 4°C until needed.

For additional information, please see our Applications Notes at http://www.med64.com/documentation

Caution: Do not touch electrodes located in the center of the MED Probes

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