In vitro culture of hiPSC-Derived Sensory Neurons: A viable human model to aid pain research and drug discovery – Supplement

Guideline for culturing neurons on multi-electrode array (MEA) platform (Alpha MED Scientific).

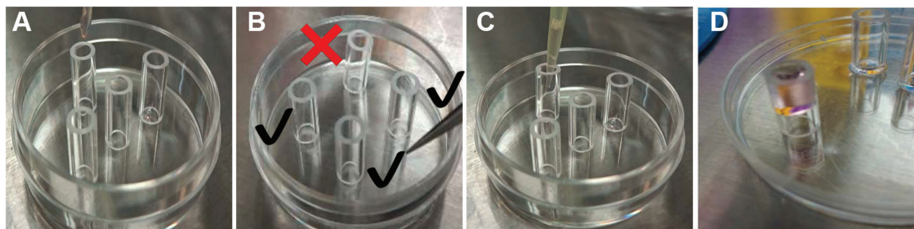
These guidelines are applicable for culturing human iPSC-Derived Sensory Neuron Progenitors and human iPSC-Derived Neural Stem Cells on MED probes for use on a MED64-Basic MEA system (Alpha MED Scientific Inc).

This application note supplement provides tips for preparing the MEA probe, thawing the cells and plating cells onto a MEA probe.

Prepare the cloning ring

- Autoclave the cloning ring (ID 3.4mm, 0.091 cm^2) prior to use to ensure sterility.
- In a biological safety cabinet, place each cloning ring upright in a sterile petri dish (**figure 1A**).
- Test if the cloning rings are stable by trying to move them. Select only the stable cloning rings (the cloning rings that were not easy to push over) (**figure 1B**). This test ensures that the cloning rings are flat, if the bottom of cloning ring is not flat, it could fall during cell seeding at the later step.
- To the cloning ring add a small amount of **Neural Plating-XF Medium** to moisten the inside of the ring (**figure 1C**). This will prevent the cell suspension from sticking within the cloning ring (**figure 1D**).

Figure 1: Prepare the cloning ring for cell seeding by standing the autoclaved cloning rings upright in a petri dish (**A**). Test if the cloning rings are stable and not easy to push over (**B**). Fill the cloning ring with **Neural Plating-XF Medium** (**C**) to prevent cell suspension becoming stuck at the top of the cloning ring (**D**).



Coating

- Pre-coat the MED-R515A probe with **950 µL** of **ReadySet** (250 µL per cm^2) and incubate at **room temperature** for **1 hour**.
- Remove the **ReadySet** solution and immediately rinse the probe **four times** with sterile ddH_2O.
- Fill the probe with sterile ddH_2O.
- Dilute the **SureBond** stock solution (50x) in Dulbecco's-PBS (1x) (D-PBS without calcium or magnesium) to make 1x working solution e.g. **120 µL** in **6 mL**. Mix well by pipetting.
- Remove the ddH_2O from the probe and immediately fill the probe with **800 µL** of **SureBond** (**figure 2C**) and incubate for **1 hour** at **37°C**.

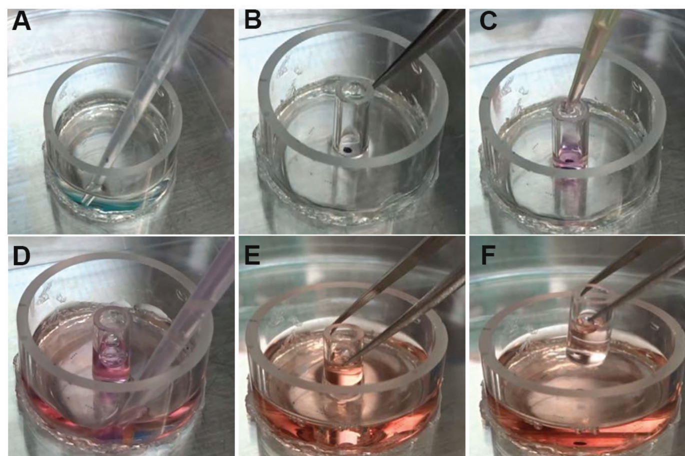
Thawing

- Thaw **Neural Plating-XF Medium** overnight at **4°C**.
- Prepare the MED-R515A probe by coating with **SureBond+ReadySet** before thawing the cells.
- Pre-warm all media and culture vessels to **37°C** before use.
- To thaw the cells – transfer the vial of cells from storage by transporting the vial buried in dry ice. Remove the vial from dry ice and transfer it to a **37°C** water bath.
- Quickly thaw the vial of cells 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**.
- Take the vial of cells to a biological safety cabinet, spraying the vial and hood thoroughly with 70% ethanol and wiping with an autoclaved paper towel before placing the vial in the hood.
- Using a P1000 pipette, transfer the cell suspension into a 15 mL sterile conical tube. Gently wash the cryogenic vial with **1 mL** of warm **Neural Plating-XF Medium** and transfer this to the 15 mL sterile conical tube.
- Add **8 mL** of **Neural Plating-XF Medium** drop-wise to the cell suspension.
- Centrifuge cells at **200 x *g*** for **5 minutes** at **room temperature**.
- Aspirate and discard the supernatant carefully with a pipette.
- Using a P1000 pipette, gently resuspend the cell pellet in **1 mL** of **Neural Plating-XF Medium** until they are in a single cell suspension.
- Perform a cell count to ensure optimal seeding density.

Plating onto MED-R515A probe

- Remove the **SureBond** coating from the MED-R515A probe (**figure 2A**).
- Place the cloning ring (ID 3.4mm, 0.091 cm^2) around the electrodes **BEFORE** the MED-R515A probe surface has dried out (**figure 2B**).
- Seed **60 µL** of cell suspension (5 x 10^5 cells/mL) into the cloning ring so that there is a final cell density of 3.0 x 10^5 cell / cm^2 (**figure 2C**).
- Pipette 1 mL of **Neural Plating-XF Medium** around the outside of the cloning ring (**figure 2D**).
- Incubate for **1 hour** at **37°C**.
- After the incubation period gently remove the cloning ring and discard (**figure 2E & F**). During the incubation period the cells will have attached onto the electrodes and will remain in this region once the cloning ring is removed.

Figure 2: Plating the cells onto MED-R515A probe. Remove the SureBond (**A**) and quickly place the cloning ring over the electrodes (**B**). Fill the inside of the cloning ring first (**C**) and then the surrounding area with **Neural Plating-XF Medium** (**D**). Remove the cloning ring gently after incubation (**E & F**) and discard.



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