NeuCyte
Translatable Neuroscience

SynFire® Human iPSC-derived induced neurons

Protocol for MED64 Presto 24-well Sakura MEA plates

Important: Before use, add the whole volume contained in each Supplement to its corresponding Basal Media bottle as follows:

1. **For the Seeding Media, add whole content of Seeding Supplement into the Seeding Basal Media bottle (Red labels).**
2. **For the Short-Term Media, add whole content of Short-Term Supplement into the Short-Term Basal Media bottle (Yellow labels). Protect the bottle from light.**
3. **For the Long-Term Media, add whole content of both Long-Term Supplements A and B into the Long-Term Basal Media bottle (Orange labels). Protect the bottle from light.**

Coating of MEA Plates (MED64 Presto 24-well Sakura plate)

1. Prepare and sterilize the 24-well Sakura plate according to MED64 Presto guidelines.
2. Prepare borate buffer (1L): dissolve 3.1 g of boric acid and 4.75 g of sodium tetraborate in distilled water. Adjust pH to 8.4.
3. Prepare 0.1% polyethyleneimine (PEI) solution: dilute 50% PEI stock solution 1:500 in borate buffer. Sterilize 0.1% PEI solution using a 0.22 µm filter.
4. Add 70 µl of prepared 0.1% PEI solution to each well of a 24-well MEA Sakura plate. Incubate overnight at 37°C.
5. Aspirate PEI solution and thoroughly rinse each well 4 x with 500µl of sterile water.
6. Dilute laminin stock (1mg/ml) to 20µg/ml in PBS.
7. Add 70 µl of prepared laminin solution to each well of the MED64 Presto Sakura plate and incubate for at least 1 hour at 37°C.
8. Aspirate laminin and directly plate cells.

Thawing of iN and Astroglial Cells

1. To thaw the cells, put the vial in a 37°C water bath with gentle agitation for ~2 minute. Keep the cap out of water to minimize the risk of contamination.
2. Transfer the cells into a 15ml conical tube with pre-warmed (37°C) DMEM-F12 Media. Repeat steps 1-2 for all cell types.
3. Check cell number and viability.
4. The recommended starting number of cells per well for a MED64 Presto 24-well Sakura plate is 270K (140K Glutamatergic, 60K GABAergic, and 70K Astroglia). Alternatively, the end-user can adjust the ratio of the different cell types to fit their experimental needs.
5. According to the recommended cell density, pool the adequate volumes (adjusted for 25 wells) of each cell type into a new 15ml conical tube, and centrifuge at 250 x g for 5 minutes at room temperature.

6. Aspirate the supernatant and resuspend the cells in 1.25 ml of **Seeding Media**. This volume corresponds to 50 µl/well (adjusted for 25 wells) for subsequent seeding on PEI/laminin-coated MEA plates.

Plating iN and Astroglial Cells into MEA Plates

1. Add 50 µl of pooled cell mix to each well of a previously coated 24-well Sakura MEA plate.
2. Incubate seeded MEA plate in a cell culture incubator at 37°C, 5% CO2, and 95% humidity.
3. Next day, add 250 µl of **Short-Term Media** to each well.

Maintaining Human Neural Co-cultures

1. Perform half-medium changes with **Short-Term Media** every other day.
2. At day 7 after seeding, switch medium gradually to **Long-Term Media** by aspirating 150 µl of old medium and adding 150 µl of new medium.
3. Perform half-medium changes with **Long-Term Media** once every 3 days.

Data Acquisition

Spontaneous synchronized neuronal network activity can be measured starting at 3-4 weeks after plating. Functional neural co-cultures can be maintained for assessment of neuronal activity for up to 8 weeks. Data acquisition for compound screening usually includes measurements of neuronal activity prior to compound application (baseline) and measurements after compound application (dosing). Recordings of neuronal activity using the MED64 Presto are performed on the Presto amplifier and data is acquired and analyzed using the MEA Symphony software according to the manufacturer's protocols.