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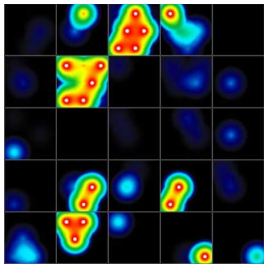
Measurement of iNeuron electrical activity using Axion Biosystems Maestro Pro MEA V.2



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We use this protocol and it's working

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Abstract

Protocol for the culture of i3N-iNeurons in CytoView MEA 96-well plates and measurements of electrical activity using the Axion Biosystems Maestro Pro MEA.

Materials

Materials/Reagents:

- iNeuron hPSC Line (iNeuron hPSC Line established on WTC11 hPSC background with TET-ON system at the AAVS1 safe-harbour locus for overexpression of murine Ngn2 and dCas9-KRAB at the CLYBL safe harbour locus was a kind gift from the lab of Michael Ward)
- mTeSR Plus (STEMCELL Technologies, 100-0276)
- Ca^{2+}/Mg^{2+}-free Dulbecco's Phosphate Buffered Saline (DPBS) (Gibco, 14190169)
- 0.5M EDTA (Invitrogen, 15575020)
- Geltrex (Gibco, A1413302)
- TrypLE Express (Gibco, 12604021)
- Dulbecco's Modified Eagle Medium (DMEM) (Gibco, 11995-065)
- Heat-inactivated foetal bovine serum (FBS) (Gibco, A5256801)
- 5mM Y27632 ROCKi (MedChem, HY-10071)
- DMEM/F12 - HEPES (Gibco, 11330032)
- Neurobasal (Gibco, 21103049)
- Glutamax Supplement (Gibco, 35050061)
- Non-essential amino acids (NEAA) (Gibco, 35050061)
- N2 supplement (Gibco, 17502048)
- B27 Supplement (Gibco, 17504044)
- Insulin (~10mg/ml, Sigma, I9278)
- B-mercaptoethanol (50mM, Gibco, 31350010)
- Accutase (Sigma, A6964)
- BrainPhys (STEMCELL Technologies, 5790)
- BDNF (Peprotech, 450-02)
- NT-3 (Peprotech, 450-03)
- Doxycycline (Dox) (Sigma, D5207)
- CytoView MEA 96-well plates (Axion, #M768-tMEA-96B)
- Axion Biosystems Maestro Pro System

Buffers/Stocks:

- Geltrex is diluted 1:50 (2x final concentration) in serum free DMEM for coating of electrodes of MEA plates.

hPSC Maintenance

1 **Maintenance of iNeuron hPSCs and iNeuron Differentiation**
Conducted as described in protocol hPSC Culture, "iNeuron Differentiation and Culture in N2B27 vs BrainPhys for Immunofluorescence and Biochemistry Assessments of Mitophagy" (https://www.protocols.io/private/E5851436F8F111EF86990A58A9FEAC02).
Specifics of the seeding and culture of iNeurons for MEA are described below in

iNeuron Culture

1m

2 **Seeding of d3 iNeurons on CytoView MEA 96-well Plate**
Before beginning make sure you have Geltrex coated the CytoView MEA 96-well plates by adding a 2x concentrated geltrex (i.e. 1:50 dilution of geltrex stock) droplet onto the centre of each well.
Be careful not to touch the edges of the wells
The hydrophobicity of the culture dish should maintain the 8 µL as a droplet encompassing the 8x electrodes.
Incubate at 37 °C for 1-2h

Note: edge effects are quite commonly a problem. It is therefore recommended to not use the perimeter wells. Do not fill with PBS at this time however.

2.1 Harvest d3 iNeurons by accutase as already described in "iNeuron Differentiation and Culture in N2B27 vs BrainPhys for Immunofluorescence and Biochemistry Assessments of Mitophagy".

Resuspend cell pellets in small volume of N2B27.
I usually resuspend the pellet in 200 µL of media per 6-well split.

2.2 Count cells and prepare cell suspension such that 5×10^4 cells would be in an 8 µL droplet.
(i.e. 6.25×10^6 cells per ml).
Note: lower and higher densities have been trialled. Lower densities tend to reduce overall electrical activities detected. And at higher densities the propensity for cultures to detach from electrodes becomes more likely.

2.3 Gently invert the geltrex coated 96-well MEA plate (make sure not to disrupt the droplet). 1m
Place the upside down plate gently into a centrifuge
230 x g, Room temperature, 00:01:00 .
The droplets of geltrex will have collected on the plate lid.
Carefully aspirate the geltrex.

2.4 Carefully add 8 µL of the cell suspension prepared in go to step #2.2 to the centre of each geltrex coated well as a droplet.
As before, avoid touching the edges of the well.
The hydrophobicity of the non-coated area of the culture dish should maintain the 8 µL as a droplet encompassing the 8x electrodes.

Note: it is important to make sure the suspension is well mixed prior to and during seeding as the high concentration means cells can aggregate and sediment quite quickly.

2.5 Carefully add 300 µL of sterile DPBS or water to the perimeter wells to minimise evaporation effects.

2.6 Place in humidified incubator, 37 °C 5%/95% CO_2/AIR mixture for 2-3hr

2.7 After 2-3hr the cells should have attached.
Gently flood the well with 100ul of N2B27 or BrainPhys media

2.8 **d4 Onwards: Half media change**
Perform a half media change with N2B27 or BrainPhys twice a week.
I usually perform the first media change after seeding on d6, but just add 100 µL media (200 µL final culture volume).

I then perform the next change change on d9, removing 75 µL media and adding 125 µL media (250 µL final culture volume).

And then Monday or Tuesday and Thursday or Friday thereafter (125 µL half media change)

Note: also maintain sterile DPBS/water levels in the perimeter wells.

MEA Measurements

1h

3 Perform a half media change 24hr before performing any MEA measurements.

3.1 Switch on Axion Biosystems Mastero Pro MEA machine.
Open lid of MEA machine and then place plate in with lid attached (A1 = Top left).
Press load button to load plate.
Open AxIS Navigator Software.
Make sure MEA incubator is on: 37 °C 5%/95% CO_2/AIR mixture
Double click on plate layout and set accordingly.
Note you can turn off electrodes in unused wells (i.e. external wells) to reduce file size.

Settings for acquisition:
Analog mode settings: Neural Spikes
Sample Frequency: 12.5kHz
Noise Subtraction / Referencing Method: Median
Digital Low Pass Filter: 3 kHz Kaiser Window
Digital High Pass Filter: 200 Hz IIR

Allow plate to equilibrate in the machine 15min prior to beginning data acquisition (it has been noticed that electrical activity is high initially but starts to reduce as cells become stabilised)

Acquire data in "AXIS Raw" file format.

MEA Analysis

4 Analyse MEA data using AxIS Navigator software

Perform "Spike Detection" using below settings:
Adaptive Threshold Crossing: 6xStd Dev
Detect Only Crossings
Pre-Spike: 0.84ms
Post-Spike: 2.14ms
Coincident Event Removal: Occurance Threshold=4, Event Window=80µs

Protocol references

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