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IPSC Neuron Development

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We use this protocol and it's working

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Abstract

IPSC Neuron Development

Materials

E8 medium (Thermo Fisher, A1517001)
Vitronectin (Thermo Fisher, A14700)
Matrigel (Corning, 354262)
Accutase (Fisher Scientific, A1110501)
E6 media - (ThermoFisher, A1516401)
Y-27632 - (Bio-Techne, 1254/10)
SB (R&D #1614)
LDN (Stemgent, 04-0074)
XAV (R&D , 3748)
N2 supplement (Stem Cell Technologies, 07156)
B27 (Fisher Scientific,17-504-044)
NAEE (Fisher Scientific,11-140-050)
Glutamax (ThermoFisher, 35050061)
PenStrep (ThermoFisher, 15140122)
Poly-L-ornithine (Sigma Aldrich, P4957-50ML)
GDNF (Recombinant Human Glial Derived Neurotrophic Factor) (Peptrotech, 450-10)
BDNF (Recombinant Human/Mouse BDNF) (R&D, 248-BDB)
AA (Ascorbic Acid) (Sigma, 4034-100g)
dbCAMP(Cyclic Monophosphate Sodium Salt)(Sigma,D0627)

before day -1

1 hESCs were cultured on 6-well coated with Vitronectin (1:100 diluted in DPBS and coated in cold room overnight) and maintained in E8 medium (Thermo Fisher, A1517001).

2 The E8 medium was changed daily, and the cells were split every 3-5 days at 70-85% confluence. then seeded into the matrigel plates for day -1

Day-1 (Cortical differentiation (H9 /E8-E6)) 1d

3 Thaw a 10mL Matrigel container overnight on ice, then aliquot & freeze. 12h

4 Dilute Matrigel 1:100 in cold DMEM/F12 and coat a 24 well with 1/2ml per well overnight at 4°C 12h

5 Aspirate E8 medium from hESCs and wash 1X with PBS. Add sufficient Accutase to cover the surface of the cells. In a 6-well plate, this is about 1mL of Accutase.

6 Incubate the plate at 37°C for 10 minutes. Then, triturate the cells in Accutase 10X using a P1000 to break them into single cells. Collect the cells into a 15mL tube, leaving one well for maintaining.

7 Spin down the 15mL canonical tube at 300g for 5 minutes. Aspirate the supernatant, and re-suspend the pellet in 1mL of E6 medium.

8 Count the cells by taking out 10uL of the re-suspended cells and mixing it thoroughly with 10uL of trypan blue in a separate 1.5mL Eppendorf tube. Load 10uL of this mixture onto a counting plate to count the cells. When you count, it will give you a concentration. Based on this concentration, plate approximately 100,000 cells/cm^2. For instance, in a 6-well plate, this is approximately 1,000,000 cells per well, and so you would need N = 1,000,000 cells/well x 6 wells = 6 million cells.

9 Using V = N/C, calculate the volume, V, needed from the 15mL tube of re-suspended cells for seeding. In a 50mL falcon tube, **E6 media+ Y-27632** (10 µM, 1:1000) + the appropriate volume (V) from the 15mL tube.

10 Add 2mL of the mixture from the 50mL plate onto the geltrex or matrigel new well plates.

Day 0 to day 3

11 Check the cell confluence. The cells need to be 100% confluent before initiating differentiation.

12 Feed cells daily with E6 media supplemented as follows with: SB (10µM - 1:1000 of the stock, stock at 10mM); LDN (100nM - 1:5000 of the stock, stock at 500 µM); XAV (500nM 1:2000 of the stock, stock at 1mM)

Day 4 to day 10

13 Feed cells daily with E6 media supplemented with: SB (10µM - 1:1000 of the stock, stock at 10mM);
LDN (100nM - 1:5000 of the stock, stock at 500 µM)

Day 10 to day 20

14 Feed cells daily with Neurobasal media supplemented with the following: N2(1:100) + B27(1:50)+NAEE+GluMax (1:1000)+P/S (1:1000)

D18 to D19 Prepare PO/LM/FN plates

1d 16h

15 Prep the plates with a poly-L-ornithine solution diluted in DPBS for 24 hours. 1d

16 Aspirate the poly-L-ornithine solution, and wash each well twice with 0.5 mL DPBS.

17 Add 12 µL Laminin (LM, 1 µg/mL) and 12 µL Fibronectin (FN, 1 µg /mL) to 12 mL DPBS. Mix and add 0.5 mL of the mixture to each well. Incubate at 37°C for ≥ 16 h. 16h

Day 20 - Passage Cells

20m

18 Wash each well with DPBS then add Accutase for 20 min / 37° C in the incubator. Follow the steps from 6-9 to re-plate the cells. However, plate the cells at a density of 150, 000 cells/cm2 in the pre-prepared PO/LM/FN plates, and use the following media mixture to feed the cells instead of E6: Neurobasal media supplemented with N2(1:100) + B27(1:50)+NAEE+GluMax (1:1000)+P/S (1:1000)+ ROCK inhibitor (1:1000, Y-27632). 20m

Day 21

19 Feed the cells on with Neurobasal basic media supplemented with: N2(1:100) + B27(1:50)+NAEE+GluMax (1:1000)+P/S (1:1000)+ ROCK inhibitor (1:1000, Y-27632) + GDNF (10ng/ml, 1X1000 dilution from 10ug/ml stock)+BDNF (10ng/ml, 1X1000 dilution from 10ug/ml stock) + AA (100uM, 1X1000 dilution from 100mM stock) + dbCAMP (100uM, 1×1000 dilution from 100mM stock).

Day 30 - end point

20 Maintain in the same media mentioned in day 21 changing ½ of the media every 5 days approx.