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Generation of human iPSC-derived cortical neurons, astrocytes, and microglia

In 1 collection

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We use this protocol and it's working

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Abstract

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1 The control neuronal precursor cell (NPC) lines were previously generated and characterized (Ref main text n 8, 86)

2 For developing NPCs, select first iPSC colonies and culture them for 4 days in embryoid body (EB) medium containing 20% KO serum replacement, 80% DMEM/F-12, 1% nonessential amino acids (NEAAs), 1% penicillin–streptomycin (PS), 10 µM SB431542 (SB), and 2.5 µM dorsomorphin.

3 Beginning on day 5, culture EBs for 4 additional days in N2B27 medium containing 100% DMEM/F-12, 1% N2, 1% B27 without vitamin A, 1% NEAAs, 1% PS, 10 µM SB, 2.5 µM dorsomorphin, and 20 ng/mL FGF2.

4 Isolate neural rosettes with Accutase, replate on Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, and maintain in N2B27 medium.

5 Manually dissect secondary or tertiary rosettes to purify NPCs.

6 Maintain NPCs in basal NPC medium consisting of 1:1 DMEM-Ham's F-12 and Neurobasal medium, 0.5% N2, 1% B27 without vitamin A, 1% PS, and 1% GlutaMAX, supplemented with 3 µM CHIR99021, 0.5 µM purmorphamine, and 150 µM ascorbic acid.

7 Split NPCs into Matrigel-coated wells every 5–6 days at a 1:10 ratio.

Cortical neuron differentiation

8 Seed NPCs at 80% confluence in neuronal differentiation medium consisting of basal neuronal medium (1:1 DMEM-Ham's F-12 and Neurobasal medium), 0.5% N2, 1% B27 without vitamin A, 1% PS, and 1% GlutaMAX, supplemented with 200 µM ascorbic acid, 1 µM purmorphamine, and 100 ng/mL FGF8.

9 Culture for 7 days, then split and seed in neuronal maturation medium consisting of basal neuronal medium supplemented with 20 ng/mL BDNF and 1 mM dibutyryl-cyclic AMP (dcAMP).

10 Replace maturation medium every other day.

11 Perform a final split after 14 days in vitro (DIV).

12 Conduct experiments after 21 DIV.

Astrocyte differentiation

13 Seed NPCs at 15,000 cells/cm² in commercial astrocyte differentiation medium (ScienCell Research Laboratories).

14 Passage cells every 5–6 days at 15,000 cells/cm² using Accutase and plate on Matrigel-coated wells in differentiation medium.

15 After 30 days, replace medium with astrocyte maturation medium. (Ref 87 main text)

16 Split cells at 90% confluence, maintain in astrocyte maturation medium, and use for experiments between passages 3 and 10 after the medium change.

Microglia differentiation

17 Seed 3 × 10^6 iPSCs into a well of an AggreWell 800 plate to form EBs.

18 Culture EBs for 4 days in mTeSR™ Plus medium supplemented daily with 50 ng/mL BMP4, 50 ng/mL VEGF, and 20 ng/mL SCF.

19 Transfer EBs to 6-well plates (15 EBs/well) and culture in X-VIVO15 medium supplemented with 1% GlutaMAX, 1% PS, 100 ng/mL M-CSF, 25 ng/mL IL-3, and 0.055 mM β-mercaptoethanol.

20 Add fresh medium weekly.

21 After 3–4 weeks, harvest macrophage precursors appearing in the supernatant.

22 Plate harvested macrophage precursors at 100,000 cells/cm² and culture for 10 days in advanced DMEM/F12 medium supplemented with 1% N2, 1% GlutaMAX, 1% PS, 100 ng/mL M-CSF, 100 ng/mL IL-34, 10 ng/mL GM-CSF, and 0.055 mM β-mercaptoethanol.