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NPC to Astrocyte Differentiation

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Neurodegeneration Method Development Community
Tech. support email: **ndcn-help@chanzuckerberg.com**

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Here, we provide a detailed protocol for differentiation of human induced pluripotent stem cell derived neural progenitor cells into astrocytes.

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Preparation

1 Prepare astrocyte medium (ScienCell Astrocyte Medium #1801) by adding FBS, antibiotic, and astrocyte growth supplement to basal media. All components are provided in ScienCell Astrocyte Medium #1801 kit.

Note: Fully prepared media should be used within two weeks when stored at **4 °C** , but can be aliquoted and stored at **-20 °C** for several months. Alternatively, aliquot the supplements and prepare smaller volumes of complete media, if desired.

2 Prior to starting, NPCs are cultured in one well of a six well plate until they reach 70% confluency

NPC to Astrocyte Differentiation - Day 1 3m

3 Prepare wells/plates/dishes to be used for the differentiation process with Matrigel

For reference, we coat a single well of a six well plate with **1 mL** of Matrigel

4 Remove media from NPCs and replace with **3 mL** Accutase

5 Incubate at **37 °C** for **00:03:00** 3m

6 Tap the plate to dissociate the cells and add **6 mL** of room temperature DMEM/F12 and collect cells into a **15 mL** tube

7 Count the number of cells and move the required volume to a new **15 mL** tube. Use the table below as a guide for the number of cells to plate into specific vessels.

Plate Size	# of Cells	Volume of Media
6-well plate	60,000/well	2mL/well
24-well plate	15,000/well	500uL/well
10cm dish	360,000	10mL

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8 Centrifuge the required volume at 750-800 rpm for 5 minutes

9 Aspirate the supernatant and resuspend the cell pellet in NPC medium

NPC to Astrocyte Differentiation - Day 2 3m

10 Remove NPC media and replace with equivalent volume of complete astrocyte medium

NPC to Astrocyte Differentiation - Day 4 and Onward

11 Change media every 2-3 days for 30 days and passage with Accutase, centrifuge at 750-800 rpm for 10 minutes and re-plate on a Matrigel coated plate whenever culture becomes 100% confluent

12 Within the first 30 days, keep the number of cells per well low to aid in differentiation

13 After 30 days, cells should be >98% positive for S100b. Some lines may express high levels of GFAP, others will not.

After full differentiation, cell confluency/number at passage is no longer important.

Astrocyte Freezing

14 Dissociate cells with Accutase and collect in complete astrocyte medium in a 15mL conical tube

15 Spin cells at 750-800 rpm for 10 minutes

The slower spinning speed helps to reduce the percentage of cell death

16 Aspirate media from pellet and resuspend in freezing media (50% complete astrocyte media,

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40% FBS, 10% DMSO)

17 Transfer cell suspension to cryovials and place in -80C for 24-48 hours before transferring to liquid nitrogen for long-term storage

Astrocyte Thawing

18 Prepare wells/plates/dishes to be used for the differentiation process with Matrigel

For reference, we coat a single well of a six well plate with **1 mL** of Matrigel

19 Add 9mL of complete astrocyte medium to a 15mL conical tube

20 Thaw frozen vial by swirling in water-bath at 37C for 60-90 seconds

21 Add thawed cells to 15mL conical tube containing astrocyte medium

22 Spin conical tube at 750-800 rpm for 10 minutes to pellet cells

23 Aspirate media from cell pellet and replace with 2mL of complete astrocyte medium

24 Transfer cell suspension to Matrigel coated plate and incubate at 37C

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