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Astrocyte generation

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We use this protocol and it's working

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Abstract

Protocol to generate astrocytes from cortical NPCs using a comercial media (AM)

NPC generation

1 To prepare the iPSC's for neural induction, iPSCs need to grow to 90 - 100% confluence.

2 As soon as the cells reach 90% confluence mTeSR media is switched to neural induction media (N2B27 supplemented with 10uM SB431542 and 1uM Dorsomorphin).

3 Neural induction media is replaced every day.

4 After 10 days of neural induction a uniform neuroepithelial sheet should appear. 500ul of 10mg/ml dispase stock solution is added directly to the media of the well to break this sheet.

5 The plate is left at 37°C until the neuroepithelial sheet lifts from the well. This can take 15-30 minutes. The sheet should come off in one piece.

6 The detached sheet can be collected gently from the well and placed into 10ml of PBS in a 15ml falcon. The cell sheet is left to sink to the bottom of the falcon tube, once this happens, PBS is removed and new PBS is added to the tube to wash the cells from remaining dispase. This PBS wash is repeated twice.

7 After the washes, PBS is removed and 2mL of neural induction media containing 10uM Y-27632 is added to the cells.

8 The cell sheet is gently broken into around 15 smaller clumps by inverting the tube, trying not to break the cell clumps up too much as this can cause non-neuronal differentiation.

9 Cells are plated onto geltrex coated wells (pre filled with 2ml of the media) in a ratio of 1:2 and placed in the incubator overnight to allow the cells to attach.

10 Media is replaced with neural maintenance media (N2B27) the day after next very gently.

11 Neural maintenance media is changed every other day. Neural rosette structures should become obvious around day 15 after neural induction.

12 When cells become confluent, typically around day 20, or if the cells are too clumpy, cells should be split and expanded further by passaging with dispase as done previously.

13 Between days 20-30 substantial neurogenesis should occur. When neurons have accumulated at the peripery of the rosettes, cells should be passaged using neat Accutase into single cells. This tends to occur close to day 30.

14 Cells are washed with PBS and 0.5 ml of Accutase per well of a 6 well plate is added. The plate is then incubated for 5 minutes at 37°C.

15 Cell clumps are dissociated into a single cell suspension by pipetting them up and down 3 or 4 times with a P1000 in the Accutase solution.

16 The Accutase solution containing the cells is diluted with 4 volumes of neural maintenance media and centrifuged at 300g for 3 mins at room temperature.

17 Supernatant is discarded and the cells are resuspended in neural maintenance media containing 10uM Y-27632 and plated in onto geltrex coated plates.

18 Media si replaced the following day with neural maintenance media.

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19 Media is switched to AM media the following day.

20 AM media is replaced every other day.

21 When cells reach 80% confluence they are split using Accutase as previously described.

22 Cells are plated at a 15K cells/cm2 in AM media. Cells need to be split following the previous steps every 7-10 days at the same density (15K cells/cm2).

23 After 21-25 days in AM media, astrocyte-looking cells start to become obvious.

24 Astrocytes can then be maintained following the same steps - passages every 7-10 days at 15K cells/cm2 in AM - for around 100 days.