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i3N neuronal differentiation

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We use this protocol and it's working

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Abstract

This protocol is based on the protocol from the Kampmann lab (Chen et al., 2020). It describes the how to differentiate i3Ns, iPSCs with stably integrated doxycycline-inducible Ngn2

Troubleshooting

Induction - day 0

1 iPSCs are maintained on Geltrex in mTeSR (STEMCELL) , and passaged using [M] 0.5 micromolar (µM) EDTA. They need to be 70-80% confluent, preferably at a low passage number when starting an induction

2 Coat 6-well cell culture plates or T75 flasks with Geltrex and incubate at 37 °C for 01:00:00

3 Prepare **pre-differentiation media** supplemented with [M] 10 micromolar (µM) **Rock inhibitors**

Note

How to prepare 50mL **Pre-differentiation media:**

- 49.8 mL Knockout DMEM/F12 (Thermo Fisher)
- 500 µL 100X N2 Supplement (Thermo Fisher)
- 500 µL Non-essential amino acids (Thermo Fisher)
- 50 µL BDNF (From 10ug/mL stock)
- 50 µL NT-3 (From 10ug/mL stock)
- 50 µL Laminin (From 1mg/mL stock)
- 50 µL Doxycyclin (From 2mg/mL stock)

4 Wash iPSCs with PBS, add 500ul of Accutase per well and incubate at 37 °C for 00:05:00 until cells have dissociated. Gently tap the plate if they are not detaching

5 After cells have dissociated, lift cells by adding 1mL of Knockout DMEM/F12 in well and pipetting up and down 2-3 times. Add cell suspension to a 15mL Falcon tube containing 4 mL of Knockout DMEM/F12

6 Spin cell suspensions at 300xG for 00:05:00

7 Aspirate the supernatant and resuspend the cells in 2 mL of **Pre-differentiation media**. Pipette gently up and down to ensure a single-cell suspension.

8 Count cells and prepare cell solution in **pre-differentiation media**. Plate 500k cells per well of a 6-well plate or 3-5 million cells in a single T75 flask.

Days 1 - 2

9 **Day 1**: remove media and add fresh **pre-differentiation media** (without Rock inhibitors from this point)

10 **Day 2:** remove media and add fresh **pre-differentiation media**

10.1 **Day 2:** Prepare plates for final plating. Coat plates with PLO (100ug/mL) in Borate Buffer. Incubate plates overnight at 37 °C

Day 3

10m

11 Finish coating the plates: Wash 2x with ddH20. Leave to dry under the hood for 00:10:00 and coat with 100ug/mL Laminin in DMEM. Incubate at 37 °C for at least 02:00:00

10m

12 At this point the cells should appear more neuronal, with neurites. If cells are healthy, prepare for plating or freeze down.

13 Prepare **neuronal media** supplemented with **doxycyclin** (100ug/mL) and [M] 10 micromolar (µM) **Rock inhibitors**

Note

How to prepare 50mL **neuronal media**:

- 23.4 mL DMEM/F12 (Thermo Fisher)
- 23.4 mL Neurobasal-A (Thermo Fisher)
- 500 µL Non-essential amino acids (Thermo Fisher)
- 250 µL 100X Glutamax (Thermo Fisher)
- 250 µL 100X N2 supplement (Thermo Fisher)
- 500 µL 50X B27 supplement (Thermo Fisher)
- 50 µL BDNF (From 10ug/mL stock)
- 50 µL NT-3 (From 10ug/mL stock)
- 50 µL Laminin (From 1mg/mL stock)

14 Wash cells with PBS and add Accutase (500 µL in 6-well plate or 4 mL in T75 flask), incubate at 37 °C for 00:05:00 until cells have dissociated

15 After cells have dissociated, lift cells by adding 1mL of DMEM+Neurobasal solution in well and pipetting up and down 2-3 times. Add cell suspension to a 15mL Falcon tube containing 4 mL of DMEM+Neurobasal

16 Spin cell suspensions at 300xG for 00:05:00

17 Plate cells into PLO-coated plates in **neuronal media** supplemented with **Doxycycline and Rock inhibitors**

Note

When plating, prepare a cell suspension of roughly 500k cells/mL of neuronal media. Depending on the platform used for the desired experiments (140 µL for 96-well plates, 1 mL of 12-well plates...)

Day 4 - onwards

18 **Day 7:** remove half of the media and add equal volume of fresh **neuronal media** (without Rock inhibitors and Doxycyclin from this point)

Note

The cells tend to become **very peely** from this point, proceed with care when handling

19 **Day 14:** remove half of the media and add equal volume of fresh **neuronal media**

Note

Cells are considered mature and are ready for experiments from Day 14.

20 **Day 21:** remove half of the media and add equal volume of fresh **neuronal media**

21 **Day 28:** remove half of the media and add equal volume of fresh **neuronal media**

Protocol references

Chen et al., 2020 dx.doi.org/10.17504/protocols.io.bcrjiv4n