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Differentiation of hiPSC-derived microglia-like cells

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We use this protocol and it's working

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Abstract

Protocol for generation of microglia-like cells from hiPSCs, based on protocol from Garcia-Reitboeck, Pablo et al., 2018.

Day 0 - Embryoid Body generation

8m

1 hiPSCs are maintained on Geltrex (ThermoFisher) in mTeSR (STEMCELL), and passaged using [M] 0.5 Mass Percent EDTA.

2 Once hiPSCs are healthy and ~80% confluent, incubate with 1 mL Accutase (STEMCELL) for 00:05:00 at 37 °C Dissociate using 1 mL of PBS and resuspend in a Falcon tube in 5 mL of PBS. 5m

3 Centrifuge at 300xg for 00:03:00 and aspirate supernatant. Resuspend pellet in 1 mL of **Embryoid Medium** (1 mL of media per well of hiPSC initially dissociated).

Note

How to prepare **Embryoid Medium:**

- mTeSR (STEMCELL)
- [M] 10 micromolar (µM) Rock Inhibitors (Tocris)
- 50 ng/mL BMP-4 (Peprotech)
- 50 ng/mL VEGF (Peprotech)
- 20ng/mL SCF (Peprotech)

4 Count cells and dilute in Embryoid medium to have suspension at 100,000 cells/mL.

5 Add 100 µL of Embryoid Medium to each well of a low-attachment U-bottom 96-well plate.

6 Using a multi-channel pipette, add 100ul of cell suspension in each well of the 96-well plate.

Note

A full-well of embryoid bodies is preferable to ensure sufficient quantity of microglia-like cells downstream, therefore ~1 million hiPSCs are needed per induction.

7 Centrifuge the plate at 800rpm for 00:03:00 and transfer to 37 °C 5% CO2 incubator. 3m

8 After 48:00:00 , supplement each well with 50 µL of fresh Embryoid media.

Day 4 - Embryoid Body Suspension and Myeloid differentiation

9 On day 4 after the start of the induction, collect every single Embryoid Body from each well and transfer in a single 15mL Falcon using a P1000 pipette and wide-bore tips.

10 Let EBs settle naturally at the bottom of the tube and aspirate Embryoid media, and Add 5mL of **Myeloid Differentiation media.**

Note

How to prepare **Myedloid Differentiation Medium:**

- X-Vivo 15 (Lonza)
- [M] 1 % volume Glutamax (ThermoFisher)
- [M] 0.5 % volume Penicilin-Streptomycin (ThermoFisher)
- [M] 50 micromolar (µM) 2-Mercaptoethanol (ThermoFisher)
- 100ng/mL MSCF (Peprotech)
- 25ng/mL IL3 (Peprotech)

11 Add 10 mL of Myeloid differentiation media to a T75 flask, and transfer the 5 mL with the EBs inside the flask for a total of 15 mL of media.

12 For the following 2 weeks (until day 18), change media with fresh Myeloid Differentiation media. Change by removing ~ 10 mL of media from the flask and replacing it with fresh media. Be careful to move the plate gently: the EBs need to stick to the flask as this will improve the microglia-like cells yield.

Day 18 onward - Microglia Harvest

13 Starting on day 18 microglia-like cells can be harvested weekly by taking 10-12mL of media from T75 flask inside a 15mL Falcon. Replace with fresh myeloid differentiation media in flask.

14 Centrifuge harvested cells in media at 300xg for 00:03:00 . Resuspend pellet in 1 mL of **Maturation Medium.**

Note

How to prepare **Maturation Medium:**
- X-Vivo 15 (Lonza)
- [M] 1 % volume Glutamax (ThermoFisher)
- [M] 0.5 % volume Penicilin-Streptomycin (ThermoFisher)
- 100ng/mL MSCF (Peprotech)

15 Filter resuspended cells through a 40-micron filter, and count. Dilute to a concentration of ~300,000 cells/mL in Maturation media.

16 Plate microglia-like cells directly onto plasticware (well-plates, imaging chambers...) in maturation media at desired concentration.

Note

The first harvest tends to give a low yield of cells. Typically, from one induction it is possible to obtain roughly 5-6 weekly harvests before the yields decrease.

17 Perform experiments on microglia-like cells after 7 days from harvest.

Protocol references

Garcia-Reitboeck, Pablo et al. “Human Induced Pluripotent Stem Cell-Derived Microglia-Like Cells Harboring TREM2 Missense Mutations Show Specific Deficits in Phagocytosis.” *Cell reports* vol. 24,9 (2018): 2300-2311. doi:10.1016/j.celrep.2018.07.094