iPSC-derived microglia protocol

Part A: For differentiation of human ES or iPS cells into hematopoietic progenitor cells

- Preparation of Media

Two medium formulations are required for the hematopoietic differentiation protocol: Medium A (Stage 1; Days 0-3) and Medium B (Stage 2; Days 3-12).

1. Thaw STEMdiff™ Hematopoietic Basal Medium at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.
2. Thaw Supplement A or B at room temperature (15 - 25°C) or at 2 - 8°C until just thawed. Mix thoroughly. If necessary, centrifuge for 30 seconds to remove liquid from the cap. After thawing aliquots, use immediately. Do not re-freeze.
3. Add Supplement A to 45mL Basal Medium and Supplement B to 75mL Basal Medium. Mix thoroughly.

- iPSC→HPC

Stage 1

D-1

1. Wash iPSC with PBS and add 1mL ReLeSR per well at RT for 30sec. Then remove and incubate for another 5 min.
2. Add 1mL mTeSR per well and firmly tap the plate to detach colonies.
3. Count # of aggregates.
    a. As an aid, before plating in 96wp, draw a cross on the wells, count in the ¼ section the # of aggregates (only count those greater than 50μm long), then multiply by 4 to get the average # of aggregates for the well.
    b. Prepare 96wp with 40μL mTeSR in the necessary wells of a 96wp.
    c. Add 10μL aggregate/well, with microscope, verify 50-200 clumps/5 μL mixture.
4. Harvest and seed human iPS cell colonies as small aggregates in mTeSR. On Day 0 (after confirming the number of adhered colonies is within 4 - 10/cm^2).

D0

1. Confirm that 16 - 40 colonies/well are adhered to the cultureware (4 - 10 colonies/cm^2).
2. Aspirate medium from wells. Add 2mL of Medium A per well (6wp).
3. Incubate at 37°C for 2 days.

D2

1. Gently remove 1mL of medium from each well. Discard.

2. Gently add 1mL of Medium A per well.
3. Incubate at 37°C for 24 hours.

Stage 2

D3

1. Aspirate medium from wells. Gently add 2mL of Medium B per well.
2. Incubate at 37°C for 2 days.

D5

1. Gently remove 1mL of medium from each well. Discard.
2. Gently add 1mL of Medium B per well.
3. Incubate at 37°C for 2 days.

D7

1. Gently remove 1mL of medium from each well. Being careful not to disturb the floating cell population. Discard.
2. Gently add 1mL of Medium B per well.
3. Incubate at 37°C for 3 days.

D10

1. Gently remove 1mL of medium from each well. Being careful not to disturb the floating cell population. Discard.
2. Gently add 1mL of Medium B per well.
3. Incubate at 37°C for 2 days.

D12

Harvest hematopoietic cells

1. Harvest supernatant cells: Using a serological pipette, vigorously pipette the cells up and down in the well to break them up as needed.
2. Transfer the cell suspension to a collection tube.
3. Add 1 mL of DMEM/F-12 per well. Triturate vigorously in the well and add to the collection tube.
4. Repeat step 3.
5. Centrifuge the collection tube at 300 x g for 5 minutes at room temperature (15 - 25°C).
6. Remove and discard the supernatant.
7. Resuspend cells pellet and count cells.
8. Seed 0.2M cells per well of 6wp in 2mL Microglia Differentiation Medium.

Part B: Differentiation and maturation kits for generation of microglia from iPSC-derived HPC

- HPC→iMGL

D0

Harvest suspended hematopoietic progenitor cells (D12 of the differentiation of iPSC into HPC protocol). Count cells using

1. Add 0.2M cells to one well of 6wp containing 2 mL Microglia Differentiation Medium.
2. Incubate at 37°C for 2 days.
3. Feed the cells every other day by topping up the well with half of the start volume of Microglia Differentiation Medium
4. Do not remove the existing medium.

D12

1. Transfer the entire cell suspension to a 50 mL conical tube.
2. Add 1 mL of DMEM/F-12 per well. Triturate vigorously in the well and add to the conical tube.
3. Repeat step 2.
4. Centrifuge at 300 x g for 5 minutes.
5. Transfer supernatant to the 50 mL conical tube. Using this supernatant, gently mix to resuspend and count cells.
6. Seed 0.6M cells per well of 6wp containing 1 mL fresh Microglia Differentiation Medium.
7. Incubate at 37°C for 2 days.
8. Feed the cells every other day for 12 days by topping up the well with half of the start volume of Microglia Differentiation Medium.

D24

Microglia maturation

1. Transfer the entire cell suspension to a 50 mL conical tube.
2. Add 1 mL of DMEM/F-12 per well. Triturate vigorously in the well and add to the conical tube.
3. Repeat step 2.
4. Centrifuge at 300 x g for 5 minutes.
5. Transfer supernatant to the 50 mL conical tube. Using this supernatant, gently mix to resuspend and count cells.
6. Seed 1M cells per well of 6wp containing 1 mL fresh Microglia Maturation Medium.
7. Incubate at 37°C for 2 days.
8. Feed the cells every other day by topping up the well with half of the start volume of Microglia Maturation Medium

D28-D34

9. Microglia are mature. These cells have limited capacity for expansion.