Dec 03, 2024

Differentiation of Human iPS Cells to Macrophages

The Journal of Cell Biology

DOI
https://dx.doi.org/10.17504/protocols.io.n2bvj965blk5/v1

Agnes Ferguson[1,2,3,4,5], Shawn Ferguson[1,2,3,4,5,6]

[1]Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06510, USA;
[2]Neuroscience, Yale University School of Medicine, New Haven, Connecticut 06510, USA;
[3]Program in Cellular Neuroscience, Neurodegeneration and Repair;
[4]Wu Tsai Institute Yale University School of Medicine, New Haven, Connecticut 06510, USA;
[5]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA;
[6]Kavli Institute for Neuroscience, Yale University School of Medicine, New Haven, Connecticut 06510, USA

Amanda Bentley-DeSousa
Yale University

DOI: https://dx.doi.org/10.17504/protocols.io.n2bvj965blk5/v1

Protocol Citation: Agnes Ferguson, Shawn Ferguson 2024. Differentiation of Human iPS Cells to Macrophages. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj965blk5/v1

Manuscript citation:
https://doi.org/10.1101/2023.10.31.564602

Protocol status: Working
We use this protocol and it's working

Created: December 03, 2024

Last Modified: December 03, 2024

Protocol Integer ID: 113463

Keywords: iPSC, macrophage, differentiation, human ips cells to macrophage, differentiation of human ips cell, human ips cell, macrophages this protocol, cell

Funders Acknowledgements:
Aligning Science Across Parkinson's Disease
Grant ID: ASAP-000580

Abstract

This protocol includes steps to differentiation human iPS cells to macrophages.

Day -2: Preparing iPS cells for hematopoietic differentiation

4m

1 Coat 3 wells of a 6 well plate with Matrigel for 01:00:00 at 37 °C. Prior to passaging, examine iPSCs under the microscope, the cells should be healthy and growing in tight colonies.

2 Remove the culture media and rinse with PBS.

3 Add 1 mL of 0.5 mM EDTA in PBS and incubate at 37 °C for 00:05:00. Check periodically under the microscope. Once 50 to 60% of colonies have detached, neutralize the reaction by adding 0 mL E8+Ri medium.

4m

3.1 Do not tap the plate to detach cells or pipet to lift cells off the plate, the goal is to collect intact cell aggregates.

4 Swirl the plate to collect all cell aggregates and transfer to a 50ml conical tube using a 5 ml pipet. Do not pipet up and down as the mechanical force will break down the aggregates.

5 Spin 100 x g, 00:03:00 the cells down. A slow spin is important to avoid single cells and small clumps.

6 Aspirate the supernatant and resuspend the pellet in 3 mL E8+Ri media by gently swirling the media in the tube.

7 Perform aggregate counts in triplicate to determine the average number of aggregates in 5 µL of sample:

7.1 Aliquot 40 µL of E8+Ri media into a 96-well plate then add 5 µL of aggregate suspension to each well.

7.2 While counting take note of aggregate size, if the size exceeds 100 cells/aggregate use a 1 mL pipettor and pipet up and down once to reduce their size.

7.3 In each well count the number of aggregates, average the results, and calculate aggregate concentration.

8 Determine the number of aggregates to plate.

8.1 The recommended number is 4-10 colonies /cm^2, however it is a good idea to try multiple plating densities to achieve optimal results.

8.2 Aspirate Matrigel from the plate prepared in step 1 and fill the wells with 1.5 mL E8+Ri. Plate 30 aggregates in the first well, 40 aggregates in the second well, and 50 aggregates in the third well.

9 Move the plate quickly back-and-forth, and side-to-side motions to distribute the aggregates in the wells and return to 37°C incubator.

Day -1: Preparing iPS cells for hematopoietic differentiation

10 Change media to E8 without Ri and incubate until colonies reach the correct size for differentiation.

Day 0: Hematopoietic differentiation

11 Count the colonies in each well, the ideal colony number is between 10-50 per well. Each colony should have at least 20-40 cells. If colonies are smaller, then change media to E8 and grow colonies for another 1-2 days.

12 After achieving desired colony number and size prepare Media A (2 mL base media + 10 µL supplement A).

13 Aspirate E8 from wells and add 2 mL of Media A. Incubate at 37 °C for 2 days.

Day 2: Hematopoietic differentiation 1d

14 Using a 1 ml pipette tip remove 1 mL of Medium A and discard.

15 Gently add 1 mL of fresh Medium A to each well.

16 Incubate at 37 °C for 24:00:00 . 1d

Day 3: Hematopoietic differentiation 2d

17 Prepare Medium B (2 mL base media + 10 µL supplement B)

18 Aspirate Medium A and add 2 mL Medium B per well.

19 Incubate at 37 °C for 48:00:00 . 2d

Day 5: Hematopoietic differentiation

20 Prepare Medium B (2 mL base media + 10 µL supplement B)

21 Using a 1 ml pipette tip gently remove 1 mL of medium from each well and discard.

22 Add 1 mL Medium B per well.

23 Incubate at 37 °C for 48:00:00 .

Day 7: Hematopoietic differentiation

24 Observe the cells under the microscope to check for presence of floating cells. The number of these
floating cells will keep increasing and further medium changes should be done carefully so as not to disturb these cells.

25 Keeping the dish flat, place the tip of a 1ml pipette to the side of the dish and gently aspirate 1 mL of medium and discard.

26 Gently add 1 mL per well of fresh Medium B.

27 Incubate at 37 °C for 72:00:00 .

Day 10: Hematopoietic differentiation 3d

28 Keeping the dish flat, place the tip of a 1ml pipette to the side of the dish and gently aspirate 1 mL of medium and discard. Make sure not to disturb the floating cells.

29 Gently add 1 mL per well of fresh Medium B.

30 Incubate at 37 °C for 72:00:00 . 3d

Day 12: Collection of hematopoietic progenitor cells (HPCs)

31 By day 12 there should be many floating HPCs in the wells.

32 To collect cells gently swirl the plate then aspirate the medium with a 5 ml pipet and transfer to a 50 ml falcon tube.

33 Add 2 mL RPMI medium to the wells, swirl then transfer to the 50 ml tube.

34 Repeat step 3 once more.

35 Spin 300 x g, 00:05:00 cells down.

36 Remove the supernatant and resuspend the cell pellet in Macrophage differentiation media, RPMI+ 20% FBS+100ng/ML M-CSF.

37 Incubate at 37 °C for 72:00:00 .

Day 15 and 18: Collection of hematopoietic progenitor cells (HPCs)

38 Supplement with 2 mL of Macrophage differentiation media.

Day 19: Collection of hematopoietic progenitor cells (HPCs)

39 Mature IBA1 positive macrophages ready for experiments.