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iPSC differentiation into Macrophages

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We use this protocol and it's working

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Abstract

This protocol describes iPSC differentiation into macrophages.

Attachments



[724-1820.docx](#)

18KB

Guidelines

This protocol is slight modification from the original protocol published.

Shi J, Xue C, Liu W, Zhang H. Differentiation of Human-Induced Pluripotent Stem Cells to Macrophages for Disease Modeling and Functional Genomics. Curr Protoc Stem Cell Biol. 2019;48

This original published protocol involves differentiation of iPSCs into CD34 positive hematopoietic progenitors by embryoid body generation on low attachment plates. Embryoid body generation is laborious, time consuming and costly. This protocol also requires FACS sorting of hematopoietic progenitors.

The CD34 positive progenitors can be differentiated without the embryoid body generation step and FACS sorting by using STEMdiff™ Hematopoietic Kit.

Materials

Reagents required

⊗ STEMdiff™ Hematopoietic Kit 1 Kit **STEMCELL Technologies Inc. Catalog #5310** (The kit has 3 components)

1. Base media, aliquot and freeze in $-20\text{ }^{\circ}\text{C}$ (3 mL and 5 mL aliquots).
2. Supplement A (200X), aliquot and freeze in $-20\text{ }^{\circ}\text{C}$, $15\text{ }\mu\text{L}$ aliquots (for 3 mL media).
3. Supplement B (200X), aliquot and freeze in $-20\text{ }^{\circ}\text{C}$, $25\text{ }\mu\text{L}$ aliquots (for 5 mL media).

FBS (#16140-071) MSR

⊗ Gibco™ RPMI 1640 Medium **Thermo Fisher Scientific Catalog #11875135**

⊗ Recombinant Human M-CSF **peprotech Catalog #300-25**

Note

Received M-CSF is a lyophilized powder, spin down the vial and resuspend in $100\text{ }\mu\text{L}$ of sterile water followed by $900\text{ }\mu\text{L}$ of 0.1% BSA (BSA solution is prepared in sterile PBS and filter sterilized).

⊗ Matrigel Growth Factor Reduced (GFR) Basement Membrane Matrix, LDEV-free **Corning Catalog #356230**

Note

iPSCs-notes: A18945 Gibco iPSCs are the best for this differentiation, WTC iPSCs also differentiation but with low efficiency compared to A18945 iPSCs. NGN WTC iPSCs efficiency is much lesser. I do not know exact reason why this difference between these cell lines.

- 6 well plate
- 96 well plate

iPSC differentiation into Macrophages

11m

1

Note

The differentiation protocol involves two steps.

1. iPSC differentiation into CD34 positive hematopoietic progenitors
2. Hematopoietic progenitors' differentiation into mature Macrophages

Note: This differentiation protocol requires healthy iPSCs approximately 50-60% confluency with nice tight colonies.

Day-2:

Coat 3 wells of a 6 well pates with Matrigel. (1 hour coating also works).

2 **Day-1:**

2.1 Bring iPSC maintenance to plate cell culture hood, remove the media and rinse once with PBS.



2.2 Then add  1 mL of  0.5 micromolar (μM) EDTA to the well and leave in the incubator for about  00:05:00 . By this time, you will see colonies lifting from the plates. If not leave plate in the incubator for few more minutes.

5m



Note

Note: Do not tap or pipet to lift off the cells from the plate. This protocol requires a clumps off iPSC colonies for good yield of hematopoietic progenitors.

2.3 Once the 50 to 60% of colonies come off the plate, bring plate into the hood and neutralize the reaction by adding  2 mL of E8+Ri media.



2.4 Now gently swirl the plate to mix EDTA solution containing cells and E8+Ri media. (Never pipet, this mechanical force will disrupt the clumps).

2.5 After swirling the plate pipet  1 mL of the cells with 5 ml pipet into 15 ml falcon tube.



2.6 Spin down the cells at  1 rcf, 00:03:00 .

3m

Note

This slow spin is required for avoiding single cells smaller clumps.



2.7 After this spin, take aspirate the supernatant and gently tap the cell pellet.

2.8 Then add  3 mL of E8+Ri media and tap gently once again to mix the clumps.



2.9 For counting the clump number take  5 μ L of this mix into a 96 well plate, add  100 μ L of E8 media. Gently tap the plate and count the colonies under the microscope. Repeat this in 3 wells of 96 well plate and average the clump number.

2.10 Now bring the Matrigel coated plate, aspirate the Matrigel and add  1.5 mL of E8+Ri media (3 well were coated on day 0).

Now plate 10 clumps in 1st well, 20 clumps in 2nd well and 30 clumps in 3rd well of the 6 well plate*. Shake the plate up and down and to sides, return the plate to the incubator.

Note

Since counting of clumps is arbitrary, it is never perfect. This exercise is essential till you get very good experience in plating the clumps.

3 Day 0

3.1 Count the colonies in in each well, ideal colony number is between 10-30. It doesn't have to be perfect number. Wells with as low as 5 colonies and max up to 30 can also be used. Anything above 40 should be avoided.

**Note****Note:**

1. Each clump should have 20-40 cells. If you have smaller colonies change media to E8 only and let the colonies grow for 1-2 days.
2. Above 30 clumps number differentiation may not work because mesodermal cells require space to migrate and differentiate into hematopoietic progenitors.

3.2 Choose one well with desired number of colonies and you can discontinue maintaining remaining wells.

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

3.4 Aspirate E8+Ri media and add  2 mL Media A and leave plate in incubator for 2 days. 

4 Day 2

Add  1 mL A ( 1 mL base media +  5 µL supplement A). 

5 Day 3

5.1 Prepare Media B ( 2 mL base media +  10 µL supplement B).

5.2 Aspirate media A and add  2 mL Media B. 

6 Day 5, 7, 9, 10

supplement the cell with  1 mL of Media B.

7 Day 12: Collection

7.1 By day 12 you will see a lot of floating hematopoietic progenitor cells.



7.2 Collect hematopoietic progenitor cells by gently swirling the plate with a 5 ml pipet into 15 ml falcon tube

7.3 Spin down the cells  3 rcf, 00:03:00 .

3m



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

7.5 Count the cell by using hemocytometer and plate 100,000 in one well of 6 well plate.

8 Day 15 and 18

Supplement with  2 mL of Macrophage differentiation media.

9 Day 19

you will have mature IBA1 positive macrophages ready for the experiments.