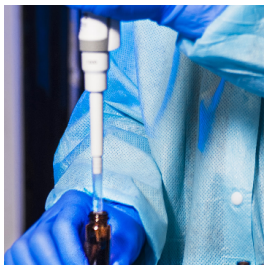
Sep 03, 2020 Version 2

# Differentiation of iPSC into Microglia-Like Cells (iMGL) V.2



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

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## Abstract

This protocol outlines the derivation of Hematopoietic Progenitor Cells and differentiation of iMGLs using iPSC cultures. This protocol is modified the following papers.

CITATION

McQuade A, Coburn M, Tu CH, Hasselmann J, Davtyan H, Blurton-Jones M (2018). Development and validation of a simplified method to generate human microglia from pluripotent stem cells.. Molecular neurodegeneration.
LINK
https://doi.org/10.1186/s13024-018-0297-x

CITATION

Abud EM, Ramirez RN, Martinez ES, Healy LM, Nguyen CHH, Newman SA, Yeromin AV, Scarfone VM, Marsh SE, Fimbres C, Caraway CA, Fote GM, Madany AM, Agrawal A, Kayed R, Gylys KH, Cahalan MD, Cummings BJ, Antel JP, Mortazavi A, Carson MJ, Poon WW, Blurton-Jones M (2017). iPSC-Derived Human Microglia-like Cells to Study Neurological Diseases.. Neuron.
LINK
https://doi.org/10.1016/j.neuron.2017.03.042

## Attachments

DOCX

Differentiation_of_i...

48KB

## Materials

Materials:

- 6-well tissue culture plate(s)
- 96-well tissue culture plate(s)
- 15 ml conical tubes
- Matrigel
- PBS
- Dispase
- Accutase
- DMEM/F12
- StemProEZPassage Disposable Stem Cell Passaging Tool
- mTesR1
- Rock Inhibitor

**Medium Recipes:**

iMGL Diff Base Medium (per 100 ml)

| | | | Vendor | Cat# | vol | |
|---|---|---|---|---|---|---|
| | phenol-free DMEM/F12 (1:1) | | Thermo Fisher | 11039021 | 92.5 | mL |
| | insulin (0.02 mg/ml) | ITS-G (100X stock) | Thermo Fisher | 41400045 | 1 | mL |
| | holo-transferrin (0.011 mg/ml) | | | | | |
| | sodium selenite (13.4 ug/ml) | | | | | |
| | B27 (2% v/v) (50X stock) | | Thermo Fisher | 17504044 | 4 | mL |
| | N2 (0.5%, v/v) (100X stock) | | Thermo Fisher | 17502048 | 0.5 | mL |
| | monothioglycerol (200 uM) | 11.5 M | Sigma Aldrich | M1753-100mL | 1.75 | uL |
| | Glutamax (1X) (100X stock) | 100X | Thermo Fisher | 35050061 | 1 | mL |
| | non-essential amino acids (NEAA; 1X) (100X stock) | 100X | Thermo Fisher | 11140050 | 1 | mL |
| | Pen/ Strep | 100X | Thermo Fisher | 15140-122 | 1 | mL |
| | (additional insulin (5 ug/mL)) we do not add it | | Sigma Aldrich | I9278-5mL | 47 | uL |

iMGL Diff Complete Medium

| | | Vendor | Cat# | dilution | |
|---|---|---|---|---|---|
| iMGL diff base medium | | | | | |
| IL-34 (100 ng/mL) | 500 ug/mL in H2O | Peprotech | 200-34 | 1:5000 | |
| TGFb-1 (50 ng/mL) | 100 ug/mL in 10mM Citric Acid | Peprotech | 100-21 | 1:2000 | |
| M-CSF (25 ng/mL) | 100 ug/mL in H2O | Peprotech | 300-25 | 1:4000 | |

iMGL Maturation Medium

| | | Vendor | Cat# | dilution | |
|---|---|---|---|---|---|
| iMGL Complete medium | | | | | |
| CD200 (100 ng/mL) | 100 ug/mL | Novoprotein | C311-50ug | 1:1000 | |
| CX3CL1 (100 ng/mL) | 100 ug/mL | Peprotech | 300-31 | 1:1000 | |

## Safety warnings

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

## Before start

**Derivation of Hematopoietic Progenitor Cells and Differentiation of iMGLs – Timeline**

1. iPSCs Culture (2-3 Days)
2. iPSCs Aggregates Plating (1 Day) *Critical: Go/No-Go Decision*
3. iPSCs Induction into Hematopoietic Stem Cells (12 Days) *Critical: Go/No-Go Decision*
4. FACs Sorting $CD43^{+}CD34^{+}$ $CD45^{+}$ Cells (1 Day)
5. Freezing Down Sorted Hematopoietic Stem Cells (1 Day)
6. Thawing Hematopoietic Stem Cells (1 Day)
7. Differentiation of Hematopoietic Stem Cells into Induced Microglia (28 Days)

## iPSCs Culture

1 Thaw and culture iPSC line per the following protocol:

Protocol

NAME

iPSC Cell Culture – Maintenance and Expansion

CREATED BY

Scott Lee

PREVIEW

1.1 To resuspend, thaw aliquot On ice .

1.2 Add 12.5 mL cold DMEM/F12.

1.3 Pipette up and down twice.

1.4 Add 1 mL of Matrigel per well of 6 well plate.

1.5 Store diluted Matrigel at 4 °C .

1.6 Prior to thawing cells, coat plate with Matrigel for 01:00:00 .

Note

1 vial of iPSC should be thawed into 1 well of a 6 well plate.

1.7 Add 9 mL DMEM/F12 to a 15 ml conical tube labeled with the iPSC line name and passage number.

1.8 Remove cells from liquid nitrogen storage.

1.9 Quickly thaw cells in 37 °C water bath and/or in hands.

1.10 Just prior to complete thaw, remove vial from water bath.

1.11 Transfer the contents of the cryo-vial (~ 1 mL) into the 15 ml conical tube.

1.12 Spin at 750 rpm for 00:03:00 at Room temperature.

1.13 Aspirate media.

1.14 Resuspend cells in 2 mL mTesR1 (supplemented with [M] 5 micromolar (µM) — [M] 10 micromolar (µM) Rock Inhibitor) by **pipetting two times**.

1.15 Transfer the cell solution to one well of a 6-well plate.

1.16 Incubate at 37 °C Overnight in **6 % $CO_2$**.

1.17 **Replace** the **media daily** until cells are ready to split or analyze.

1.18

Note

Media should be changed daily. It is okay to skip a media change one time each week if double feeding is performed; however, this is largely dependent on the density of the cells and volume of media (do **not double feed** if cells are **more than 70% confluent**).

Aspirate media.

1.19 Gently add fresh mTesR1 to cells (volume depends on cell density and well size).

- 0.5 mL per well to 24 well plate
- 2 mL — 4 mL per well to 6 well plate
- 5 mL — 10 mL to 10 $cm^2$ plate

1.20 Incubate at 37 °C in **6 % $CO_2$.**

1.21

Note

When differentiating cells appear in the culture, it is important to remove all the cells promptly.
Repeated cleaning may be necessary over the course of several days to remove all the material. If differentiation is excessive and line is precious, perform subcloning.

Under microscope, remove differentiated cells with p20 or p200 tip (depending on the amount of differentiation). Transfer the cells/media to a biohazard bag.

1.22 Gently wash cells with 1x PBS.

1.23 Add fresh mTesR1.

- 0.5 mL per well to 24 well plate
- 2 mL — 4 mL per well to 6 well plate
- 5 mL — 10 mL to 10 $cm^2$ plate

1.24 Incubate at 37 °C in **6 % $CO_2$** until cells are **60 — 80 % confluent**. Change mTesR1 media **daily** until
cells are needed. Repeat cleaning as necessary.

1.25 iPSCs grow on Matrigel. Plates should be coated with Matrigel at least 1 hour prior to plating and no
longer than 24 hours prior to plating cells:

- 0.5 mL in 12 well plate
- 1 mL in 6 well plate
- 4 mL in 10 $cm^2$ plate

Note

It is critical to keep Matrigel on ice while coating. Prior to plating cells, ensure Matrigel has not
evaporated from well.

1.26 Aspirate media.

1.27 Gently wash cells with 1x PBS (**2 — 3 ml/well**).

1.28 Add Accutase (Gibco A11105-01) directly to the cells and incubate at 37 °C for 00:03:00 — 00:04:00 .

- 6 well plate, add 0.75 mL — 1 mL per well
- 24 well plate, add 0.5 mL
- 10 $cm^2$ dish, add 3 mL

1.29 Tap dish to aid in dislocation of cells.

1.30 Add DMEM/F12 directly to cells and scrape gently to remove all cells (use p1000 for 24 well plate, and cell scraper for 6 well plate and 10$cm^2$ dish).

- 6 well plate, add 2 mL — 4 mL per well
- 24 well plate, add 1 mL
- 10 $cm^2$ dish, add 9 mL

1.31 Collect cells in conical tube (15 ml/50 ml depending on volume).

1.32 If necessary, add 2 mL — 5 mL DMEM/F12 to dish to remove all cells from the dish and add to conical tube.

1.33 Centrifuge cells at 750 rpm for 00:03:00 at Room temperature .

1.34 Carefully aspirate supernatant.

Note

To avoid aspirating cell pellet, it is OK to leave a small amount of media ( 0.5 mL — 1 mL ).

1.35 Resuspend cell pellet with mTesR1 (Rock Inhibitor addition varies, see below).

- 2 mL mTesR1 per well of a 6 well plate
- Our goal is to maintain iPSC lines without using Rock Inhibitor; however, this must be done through careful weaning off Rock Inhibitor
- All cells should be thawed in Rock Inhibitor:

- [M] 10 micromolar (µM) concentration for new iPSC lines, lines thawed from 96 well after editing.
- [M] 5 micromolar (µM) concentration if thawing from a line without knowledge of its Rock sensitivity.
- [M] 1 micromolar (µM) concentration for all other lines (for lines still exposed to Rock Inhibitor, use [M] 1 micromolar (µM) . Otherwise, do not use Rock Inhibitor.)

1.36 Pipet cells **2 times** only to preserve clumps.

1.37 Transfer cell suspension to appropriate plate (pre-coated with Matrigel for at least 01:00:00 ).

- For maintenance, dilute cells 1:3 in mTesR1
- For expansion, plate all cells

1.38 Incubate at 37 °C in **6 % $CO_2$** until cells are **60 — 80% confluent**. Change mTesR1 media **daily** until cells are needed.

1.39 Aspirate media.

1.40 Gently wash cells with 1x PBS (Use 2 mL — 3 mL per well in 6 well plate).

1.41 Add Accutase (Gibbco A11105-01) directly to the cells and incubate at 37 °C for 00:03:00 — 00:04:00 .

- 6 well plate, add 0.75 mL — 1 mL per well
- 10cm$^2$ dish, add 3 mL

1.42 Tap dish to aid in dislocation of cells.

1.43 Add DMEM/F12 directly to cells.

- 6 well plate, add 2 mL — 4 mL per well
- 10cm$^2$ dish, add 9 mL
- If cells remain attached, use a cell scraper to gently dislodge cells (apply gentle pressure and use 1 — 2 passes to remove cells)

1.44 Collect cells in conical tube (15 ml/50 ml depending on volume).

1.45 Add 2 mL — 5 mL DMEM/F12 to dish to remove all cells from the dish and add to conical tube.

1.46 Centrifuge cells at 750 rpm for 00:03:00 at Room temperature .

1.47 Carefully aspirate supernatant.

Note

To avoid aspirating cell pellet, it is OK to leave a small amount of media ( 0.5 mL – 1 mL ).

1.48 Resuspend cell pellet with mTesR1 (No Rock Inhibitor).

- Use volume appropriate for freezing
- Assume 1 mL per cryovial total and add ½ total volume of mTesR1
- Pipet cells 1 — 2 times only to preserve cell clumps

Note

**Example**: to freeze 10 tubes, you will need 10 mL total and will add 5 mL mTesR1 to cell pellet (and 5 mL of 2x Freezing Media below)

1.49 Add an equal volume of cold 2x Freezing Media (20 % DMSO, FBS). Pipet cells 1 time only to preserve cell clumps.

1.50 Transfer cell suspension to pre-labeled cryovials ( 1 mL per cryovial).

Ensure that cryovials are labeled with the following:

- Cell Type
- Line Name
- Passage #
- Date
- Your Name

1.51 Freeze vials at -80 °C in foam racks for 48:00:00 — 72:00:00 .

1.52 Transfer vials to **liquid nitrogen** for long-term storage.

## iPSCs Aggregate Plating

2 Once iPSCs are 70-80% confluent in 2-3 wells of a 6-well tissue culture plate, passage and plate the iPSCs as aggregates

Note

Aggregates should be approximately 100-200µm in diameter

3 Coat a 6-well tissue culture plate with **Matrigel** for a least 01:00:00 prior to passaging cells

Note

A single 6-well tissue culture plate will yield approximately 1-1.5 million HPCs

4 Set the following media out to warm to Room temperature :

- Dispase
- DMEM/F12
- PBS

- mTesR1

5 After plate has been coated for 01:00:00 and media has warmed to Room temperature, proceed to passage aggregates as described below:

5.1 Aspirate media from well.

5.2 Wash cells with 2 mL of PBS per well

5.3 Aspirate **PBS** from well.

5.4 Add **Dispase** to cells 1 mL per well.

5.5 Incubate at 37 °C for between 00:07:00 and 00:08:00.

Note

Edges of iPSC clusters should begin to lift off the Matrigel coated plate

Note

Cells will not completely dissociate from Matrigel coated plate. The majority of cells will remain attached after the incubation.

5.6 Aspirate the **Dispase** from the wells.

5.7 Gently wash cells 2-3 times with 2 mL of DMEM/F12 per well, aspirating after each wash.

Note

The major goal of this step is to remove the Dispase without substantially disturbing the cells.

5.8 Add 2 mL of DMEM/F12 to each well.

5.9 Using the **StemProEZPassage Disposable Stem Cell Passaging Tool**, cut the iPSCs into aggregates that are approximately 100-200µm in diameter.

Note

To accomplish this, the StemPro EZPassage Disposable Stem Cell Passaging Tool should be rolled throughout the entirety of the well horizontally (twice) and vertically (twice). This will result in the appropriate sized aggregates for plating.

5.10 Transfer the detached aggregates with a serological pipette into a 15 ml conical tube.

Note

Check tissue culture plate under microscope to ensure that the majority of aggregates have been transferred from the plate.

Note

If aggregates still remain, wash well with 2mL of DMEM/F12 and transfer to 15mL conical tube

5.11 Centrifuge 15 ml conical tubes containing cell aggregates at 750 rpm for 00:03:00 .

5.12 Aspirate supernatant from cell pellet.

5.13 Resuspend pellet gently in 2 mL of mTesR1

Note

Obtaining a uniform suspension of aggregates approximately 100-200µm in diameter is optimal. The StemPro EZPassage Disposable Stem Cell Passaging Tool should be used to ensure these aggregate sizes.

Note

It is essential that pellet is resuspended by pipetting only one time, this ensures a larger degree of cell survival – as cells are incredibly delicate at this stage.

6 Perform triplicate aggregate counts to determine the average number of cell aggregates.

6.1 Pipette 40 µL pf DMEM/F12 into three individual wells of a 96-well flat bottom tissue culture plate.

6.2 Pipette 5 µL of aggregate suspension to each well.

6.3 Manually count the number of aggregates in each well.

Note

Do not count aggregates smaller than 100µm

6.4 Calculate the average number of aggregates per well.

Note

Add the number of aggregates per well and then divide by 3 to find the average number of aggregates per well

6.5 Next calculate the *Concentration of Aggregates* or *Aggregates/uL.*

Note

Take the average number of aggregates per well and divide by 5 (the dilution factor) to obtain the number of aggregates per microliter.

7 Determine the number of aggregates to plate in a 6-well tissue culture plate.

Note

It is recommended plating 130-160 aggregates per well (optimal for control iPSC lines). This density typically results in 20-38 colonies per well after 24 hours of incubation.

Note

The number of aggregates plated can be adjusted depending on how particular iPSC lines behave.

Note

Plating more aggregates at this stage does not result in more HPCs obtained at the end of the protocol.

7.1 Calculating the plating volume of the cell aggregate mixture is accomplished by dividing the number of aggregates to plate by the concentration of the cell aggregates. For example:

1. Plate 130 aggregates per well
2. Concentration of Cell Aggregates =3 Aggregates/µL
3. 130 Aggregates per Well / 3 Aggregates per µL= Plate ~43µL of Aggregate Mixture per Well

8 After calculating the number of aggregates to plate per well, aspirate **Matrigel** from previously coated 6-well plate(s) (refer to **Step 2**), and add 2 mL of mTesR1 containing **2.5µM Rock Inhibitor** to each well.

8.1 Gently mix the cell aggregate mixture by inverting 15mL conical tube.

Note

Do not vortex or pipette mixture.

8.2 Add the previously calculated volume of aggregate suspension to each well in the 6-well plate.

8.3 Place the plate containing **mTesR1** and aggregate suspension in incubator at 37 °C for 24:00:00 .

Note

Prior to placing plate in incubator, perform a “t” shaped motion with the plate to ensure that aggregates are distributed evenly throughout the wells.

8.4 After 24 hours, carefully aspirate **mTesR1** containing **2.5µM Rock Inhibitor** from each well.

8.5 Gently wash cells with 2 mL of PBS per well.

8.6 Aspirate **PBS**.

8.7 Add 2 µL of pre-warmed fresh mTesR1 to each well.

8.8 Visualize plate under microscope and manually count the number of adhered aggregates per well.

Note

Aim for at least 20 adhered aggregates per well of a 6-well tissue culture plate

Note

Do not proceed further if < 20 aggregates or > 40 aggregates have adhered to 6-well tissue culture plate as differentiation will be compromised

## iPSCs Induction into Hematopoietic Stem Cells

9 Prepare **Medium A** per the following recipe:

1. Add Supplement A to Hematopoietic Basal Medium at a concentration of 1:200

Note

Medium A can be prepped and stored for a maximum of three days

10 Prepare **Medium B** per the following recipe:

1. Add Supplement B to Hematopoietic Basal Medium at a concentration of 1:200

Note

Medium B can be prepped and stored for a maximum of three days

11 Change media on the cell aggregates using the following schedule.

11.1 **Day 0** - Aspirate medium from wells and add 2 mL of Medium A per well.

Note

Day 0 starts 24 hours after aggregate plating

11.2 **Day 2** - Gently remove 1 mL of Medium A from each well and gently replace with 1 mL of fresh Medium A per well.

Note

It is best to use a serological pipette or a 1mL micropipette to perform the media removal.

11.3 **Day 3** - Aspirate **Medium A** from wells and gently add 2 mL of Medium B per well.

11.4 **Day 5** - Gently remove 1 mL of Medium B from each well and gently replace with 1 mL of fresh Medium B per well.

11.5 **Day 7** - Gently remove 1 mL of Medium B from each well and gently replace with 1 mL of fresh Medium B per well.

Note

At this point, floating cells can often be seen in culture and they will continue to increase in number for the remainder of the protocol.

11.6 **Day 10** - Gently remove 1 mL of Medium B from each well and gently replace with 1 mL of fresh Medium B per well.

Note

As the number of floating cells in the culture increases, it is imperative that media changes are done slowly and gently to avoid removing a large number of the floating cells.

12 **Harvesting Cells for FACS Sorting:**

12.1 Floating and adherent cells should be harvested for FACS sorting on the twelfth day of culture for presence of the following cellular markers:

1) CD43
2) CD34
3) CD45

12.2 Begin harvesting floating cells using a serological pipette or 1mL micropipette, vigorously pipette media and cells up and down approximately 2-3 times in the well to break up floating cell aggregates.

12.3 Transfer floating cells and media to appropriately sized conical tube.

12.4 Wash well with 1 mL of DMEM/F12 and transfer to same collection tube, this will ensure the majority of floating cells have been collected.

Note

Be sure that collected cells are kept on ice to avoid cell death.

12.5 Centrifuge the collection tube at 300 x g for 00:05:00 at 4 °C .

12.6 Aspirate supernatant.

12.7 Re-suspend pellet in 5 mL of sterile FACS Buffer (PBS and 2% FBS) .

12.8 Filter the suspension through a 40µm filter into collection tubes.

12.9 Centrifuge the collection tube at 300 x g for 00:05:00 at 4 °C .

12.10 Aspirate supernatant.

12.11 Re-suspend pellet in 5 mL of sterile FACS Buffer (PBS and 2% FBS) .

12.12 Begin harvesting Adherent Cells by first adding 1 mL of Accutase to each well.

12.13 Incubate at 37 °C for 00:15:00 .

12.14 Add 2 mL of DMEM/F12 to each well.

12.15 Triturate vigorously to achieve a single cell suspension.

12.16 Transfer cell suspension to appropriately sized collection tube.

12.17 Centrifuge the collection tube at 300 x g for 00:05:00 at 4 °C .

12.18 Aspirate supernatant.

12.19 Re-suspend pellet in 5 mL of sterile FACS Buffer (PBS and 2% FBS) .

12.20 Filter the suspension through a 40µm filter into collection tubes.

12.21 Centrifuge the collection tube at 300 x g for 00:05:00 at 4 °C .

12.22 Re-suspend pellet in 5 mL of sterile FACS Buffer (PBS and 2% FBS) .

## FACS Sorting CD43$^+$ CD34$^+$ CD45$^+$ Cells

13 To stain cells for FACS sorting, add the following antibodies to the filtered cell suspension (cells and FACS Buffer) in the noted concentrations:

- CD34-FITC (1:200)
- CD43-APC (1:200)
- CD45 – Alexa Fluor700 (1:200) (*optional*)

- CD41-PE (1:200) (*optional*)

14 Incubate cells and antibodies On ice in the dark for 00:20:00 .

15 After incubation, add 2 mL of FACS Buffer to each tube and centrifuge at 300 x g for 00:05:00 .

16 Aspirate supernatant.

17 Re-suspend pellet in 500 µL of FACS Buffer .

18 Sort the $CD34^+$ and $CD43^+$cell population using a **Becton Dickinson FACSAria II**.

Note

Sorting has to be performed in sterile conditions.

Note

In order to obtain high quality HPCs, it is suggested to sort only the $CD34^+$ and $CD43^+$ double positive cell population, discarding the single or double negative cells.

## Freezing Down Sorted Hematopoietic Stem Cells

19 Centrifuge positively sorted cells at 300 x g for 00:05:00 at 4 °C .

20 Aspirate supernatant.

21 Re-suspend cells at a concentration of 1 million cells per 1 mL of Cryostor CS10 .

22 Aliquot 1 mL of cell and freezing medium suspension per cryovial.

23 Place cells in -80 °C for approximately 48:00:00 .

24 After 48 hours, cells can be transferred to liquid nitrogen for long-term storage.

## Deriving iMGLs - Thawing Hematopoietic Stem Cells

25 Using previously sorted cryopreserved cells (**Freezing Down Sorted Hematopoietic Stem Cells Section**), place frozen vial of cells in 37 °C water bath for quick thaw.

Note

Thaw should take less than one minute, remove cells from water bath prior to complete thaw.

26 Transfer contents of cryovial to a conical tube containing 8 mL of DMEM/F12 supplemented with 2% FBS .

27 Centrifuge concial tube at 300 x g for 00:05:00 .

28 Aspirate supernatant.

29 Re-suspend cell pellet in iMGL Diff Complete Medium at a concentration of ~500,000 cells per 2 mL .

29.1 **iMGL Diff Complete Medium Recipe:**

| | | Vendor | Cetalog # | Dilution |
|---|---|---|---|---|
| iMGL Diff Base Medium | | | | |
| IL-34 (100 ng/ml) | | Peprotech | 200-34 | 1:5000 |
| TGFb-1 (50 ng/ml) | | Peprotech | 100-21 | 1:2000 |

| | | | | | |
|---|---|---|---|---|---|
| | M-CSF (25 ng/ml) | | Peprotech | 300-25 | 1:40 00 |

Note

*iMGL Diff Base Medium* can be kept in storage at 4°C for approximately one month. It is important to make fresh *iMGL Diff Complete Medium* (addition of fresh cytokines to the base medium) every time you need to feed the culture

## Differentiation of Hematopoietic Stem Cells into iMGLs

30 **Day 2** - Add 1 mL of IMGL Diff Complete Medium per well of a 6-well tissue culture plate.

31 **Day 4** - Add 1 mL of IMGL Diff Complete Medium per well of a 6-well tissue culture plate.

32 **Day 6** - Add 1 mL of IMGL Diff Complete Medium per well of a 6-well tissue culture plate.

33 **Day 8** - Add 1 mL of IMGL Diff Complete Medium per well of a 6-well tissue culture plate.

34 **Day 10** - Add 1 mL of IMGL Diff Complete Medium per well of a 6-well tissue culture plate.

Note

Use extreme caution as plate is nearly full with media.

35 **Day 12** - Collect all but 1 mL of media from wells and centrifuge at 300 x g for 00:05:00 at Room temperature .

35.1 Aspirate the media and add 1 mL of iMGL Diff Complete Medium per well to re-suspend cell pellet.

36 **Day 14** - Add 1 mL of IMGL Diff Complete Medium per well of a 6-well tissue culture plate.

37 **Day 16** - Add 1 mL of IMGL Diff Complete Medium per well of a 6-well tissue culture plate.

38 **Day 18** - Add 1 mL of IMGL Diff Complete Medium per well of a 6-well tissue culture plate.

39 **Day 20** - Add 1 mL of IMGL Diff Complete Medium per well of a 6-well tissue culture plate.

40 **Day 22** - Add 1 mL of IMGL Diff Complete Medium per well of a 6-well tissue culture plate.

41 **Day 24** - Add 1 mL of IMGL Diff Complete Medium per well of a 6-well tissue culture plate.

Note

Use extreme caution as plate is nearly full with media.

42 **Day 25** - Collect all but 1 mL of media from wells and centrifuge at 300 x g for 00:05:00 at Room temperature .

42.1 Aspirate the media and add 1 mL of iMGL Maturation Medium per well to re-suspend cell pellet.

iMGL Maturation Media Recipe:

| | | | Vendor | Catalog # | Dilution |
|---|---|---|---|---|---|
| | iMGL Complete Medium | | | | |
| | CD200 (100 ng/ml) | 100 ug/ml | Novoprotein | C311-50 ug | 1:1000 |
| | Cx3CL1 (100 ng/ml) | 100 ug/ml | Peprotech | 300-31 | 1:1000 |

Note

*iMGL Diff Base Medium* can be kept in storage at 4°C for approximately one month. It is important to make fresh *iMGL Diff Complete Medium* (addition of fresh cytokines to the base medium) every time you need to feed the culture

43 **Day 28** - Cells should have reached maturity by this step.

Note

Continue feeding cells with **iMGL Maturation Media**. Mature Microglia-Like Cells can be used for approximately 2-3 weeks.

Note

Mature iMGLs can be also be detached with **Accutase** ( 00:05:00 at 37 °C ) and replated on plastic or glass culture ware that has been pre-coated with **Matrigel**.

## Citations

McQuade A, Coburn M, Tu CH, Hasselmann J, Davtyan H, Blurton-Jones M. Development and validation of a simplified method to generate human microglia from pluripotent stem cells.
https://doi.org/10.1186/s13024-018-0297-x

Abud EM, Ramirez RN, Martinez ES, Healy LM, Nguyen CHH, Newman SA, Yeromin AV, Scarfone VM, Marsh SE, Fimbres C, Caraway CA, Fote GM, Madany AM, Agrawal A, Kayed R, Gylys KH, Cahalan MD, Cummings BJ, Antel JP, Mortazavi A, Carson MJ, Poon WW, Blurton-Jones M. iPSC-Derived Human Microglia-like Cells to Study Neurological Diseases.
https://doi.org/10.1016/j.neuron.2017.03.042