

The production of monocyte-derived dendritic cells from human iPSCs

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Abstract

This protocol is a modification of a previously published method by Wilgenburg et al ([PLoS One, 2013](#)) to obtain monocytes and Dendritic cells (DCs) from induced Pluripotent Stem Cells (iPSCs) lines.

Citation: Lucas Armitage, Mark Wallet The production of monocyte-derived dendritic cells from human iPSCs. [protocols.io](#)

[dx.doi.org/10.17504/protocols.io.ng8dbzw](https://doi.org/10.17504/protocols.io.ng8dbzw)

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Before start

1. Order necessary supplies

Item	Catalog #	Vendor
hES Medium		
KO DMEM	10829-018	Gibco
KO Serum replacement	10828	Gibco
NE Amino Acids	M11-003	PAA
GlutaMAX	35050	Gibco
2-Mercaptoethanol	31350-010	Gibco
bFGF	4114-TC-01M	&D Systems
Pen/Strep	P11-010	PAA
X-VIVO FACTORY base		
X-VIVO 15	BE04-418	Lonza
GlutaMAX	35050	Gibco
2-Mercaptoethanol	31350-010	Gibco
Additional Growth Factors		
M-CSF	300-25	PeptoTech
IL-3	200-03	PeptoTech
Rock inhibitor: Y-27632 dihydrochloride	1254	Tocris

GM-CSF	300-03	PeptoTech
IL-4	200-04	PeptoTech
Other		
Ultra-low attachment 6 well plates	3471	Corning from Appleton Woods/or Fisher Scientific Ltd
hESC-Qualified Matrix Matrigel	354277	Corning
mTeSR1 media	05850	Stem Cell Technologies

2. Prepare solutions

X-VIVO Factory base medium

1. 500mL X-VIVO 15
2. 5mL Glutamax (100X)
3. 500µL 2-ME (1000x)

FACTORY medium complete

1. 25 mL of basic medium
2. 250 µL MCSF / IL-3 cocktail

Stock MCSF = 20 µg/mL (final = 200 ng/mL)

Stock IL-3 = 5 µg/mL (final = 50 ng/mL)

3. Pen/Strep

DC DIFFERENTIATION base

1. 500 ml CellGenix GMP DC Medium (20801-0500) [Serum Free]

DC DIFFERENTIATION complete

1. To a 15 mL aliquot of the base medium, add 50 ng/ml IL-4 and 50 ng/ml GM-CSF (150 µl of aliquotted and mixed cytokines each at 100X [5 ug/ml]) [Do not freeze thaw cytokines. Prepare single use aliquots in CellGenix GMP DC medium and store at -80C]

Protocol

Sample

Step 1.

Start with a well of a 10 cm plate with iPS cells grown on Matrigel as follows:

A. Culture of iPSCs/ESCs on Matrigel (hES qualified) coated dishes in a 10 cm dish

📌 NOTES

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Cells require media changes every day starting on Day 1. The maintenance media is orange in color to begin with. The following is a guideline, but you can add more fresh media as necessary

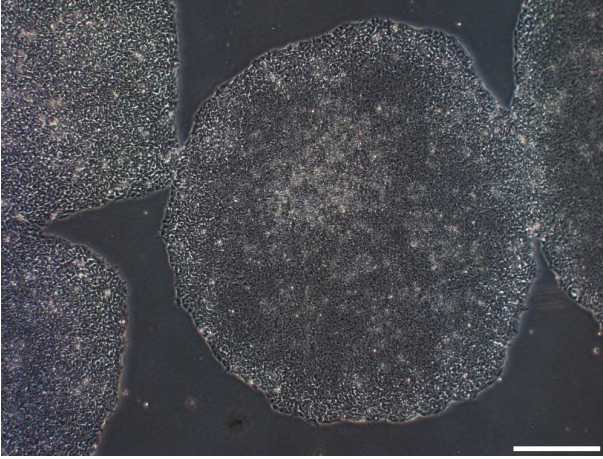
Daily Maintenance

Step 2.

- **Day 0**, Passage iPSCs onto 10 cm Matrigel coated dish in 8 mL mTesR1 media **passage clumps sparsely so that they will have room to grow into large colonies by Day 10** (**See Image 1**)
- **Day 1 Δ media**, add 8 mL fresh media
- **Day 2 Δ media**, add 8 mL fresh media
- **Day 3 Δ media**, add 9 mL fresh media
- **Day 4 Δ media**, add 9 mL fresh media
- **Day 5 Δ media**, add 10 mL fresh media
- **Day 6 Δ media**, add 10 mL fresh media
- **Day 7 Δ media**, add 11 mL fresh media
- **Day 8 Δ media**, add 11 mL fresh media
- **Day 9 Δ media**, add 12 mL fresh media

📈 EXPECTED RESULTS

Image 1: iPSC colonies just before lifting for EB culture.



Setting up Embryoid Bodies (EBs)

Step 3.

Pre-treat iPSCs for 1 hr with 10 μ M ROCK inhibitor (Y-27632) to reduce apoptosis of cells that occurs during the scraping step

Setting up Embryoid Bodies (EBs)

Step 4.

Add 2 mL fresh media (+10 μ M ROCK inhibitor) to each well of a 6-well non-adherent plate (to pre-wet the wells)

Setting up Embryoid Bodies (EBs)

Step 5.

Use a cell lifter to gently dislodge the colonies, transfer colonies and media gently to a 15 mL conical tube to allow colonies to gravity settle. Aspirate media as soon as large colonies are settled (this will help remove small colonies or single cells)

Setting up Embryoid Bodies (EBs)

Step 6.

Add 12 mL mTeSR1 + ROCK inhibitor to 15 mL conical tube with settled colonies, gently mix and distribute the volume equally among wells of the 6-well plate prepared above (total volume per well = 4 mL mTeSR1 + ROCK inhibitor (10 μ M)

Setting up Embryoid Bodies (EBs)

Step 7.

Change $\frac{3}{4}$ media on Day 2 (tip plate and allow EBs to settle to the bottom edge). Rather than using vacuum aspiration it is easier to remove media using a serological pipet. Replace with 4 mL fresh mTeSR1 + ROCK inhibitor per well

Setting up Embryoid Bodies (EBs)

Step 8.

Collect EBs on Day 4. Transfer EBs and media to 15 mL conical tube(s). Gravity settle EBs, remove

media, wash with plain XVIVO-15 media (no growth factors). (See Image 2 & 3 for examples of EBs)

✓ EXPECTED RESULTS

Image 2: EBs after 4 days culture [Note, this is an exceptionally good looking culture. Often, cystic regions will be less prominent, but differentiation still works]

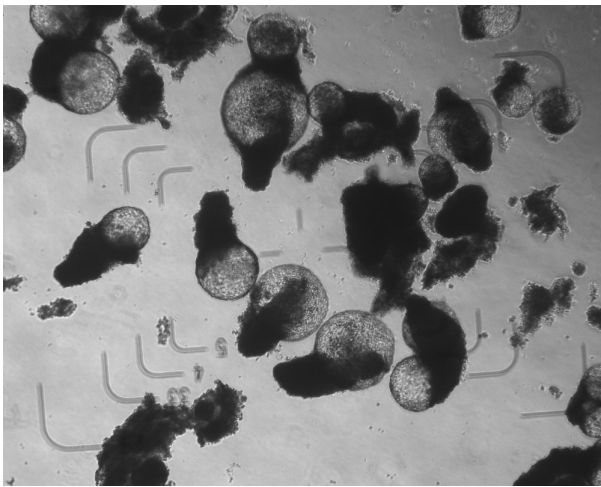
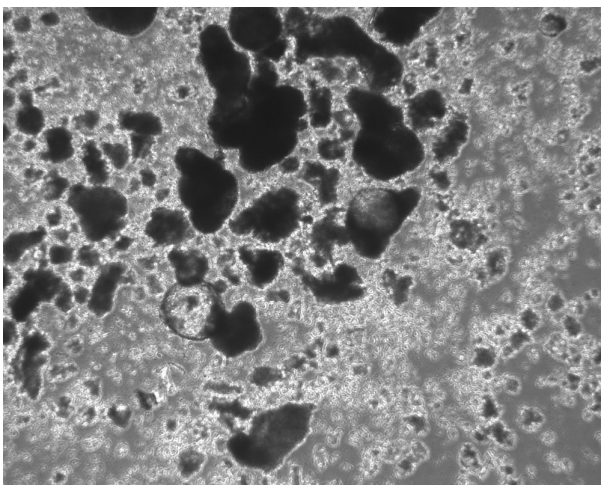


Image 3: Less photogenic EBs that still yielded quality monocytes [Note the small debris because the wash step to remove small cells/debris had not been included in the protocol at this point].



Setting up FACTORIES

Step 9.

Estimate the total number of medium to large EBs formed (this is a judgement call that becomes easier only with experience).

Setting up FACTORIES

Step 10.

Harvest EBs into a 50mL conical tube and allow to settle for a few mins. The large clumps (= good EBs) will sink, whilst individual/dead cells will remain floating. Remove supernatant, add 10ml factory base Repeat settling and wash with Factory base medium.

Setting up FACTORIES

Step 11.

Resuspend EBs in 4 ml factory complete medium for every 20 EBs. Transfer 4 mL EBs + medium into each well of an adherent, regular tissue culture 6-well plate. Aim to end up with around 15 - 20 EBs per well (too many will inhibit monocyte production). If the original stem cell well was dense the EBs may be split between two or more wells.

Setting up FACTORIES

Step 12.

Incubate for several days. If there is a lot of cell debris, then medium may need changing (warm medium, and always leave at least 1mL in the well), but otherwise do 50% approximately every 5 days or when the medium becomes yellow. Total volume can be increased to 6mL.

📌 NOTES

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EBs set up as factories in X-VIVO factory medium may not adhere to plates (most often the EBs adhere, but adherence is weak so medium exchange must be done very gently).

Harvesting monocytes for dendritic cell differentiation

Step 13.

Monocytes will start to appear in supernatant after 10 - 20 days. (**See Image 4**) The monocyte harvest described below can be performed every 5 days for approximately 4-6 weeks. The first harvest yields the fewest monocytes, but yield tends to increase for harvests 2-5. Total yield from a 6-well plate of factories can reach about 2 million monocytes per harvest.

A. Gently swirl plate then harvest 5ml of the supernatant (leaving 1ml behind) - be careful not to disrupt factory, and always use warm medium, otherwise factory may curl up. (Care is required for XVIVO factories since factories may not have adhered to plate).

B. Count cells, spin at 400g for 5mins, resuspend pellet in DC DIFFERENTIATION medium in low adherence plates (crucial that only low adherence plates are used here). Plate at 4×10^6 per well of 6 well plate, 4mL/well (or equivalent density for other well-sizes; 5×10^5 works well for 24 well plates).

C. Cells will grow loosely adherent or floating near the bottom in clusters.

D. Add 2 ml of DC DIFFERENTIATION medium at day 3.

E. Remove 2 ml of DC DIFFERENTIATION medium on day 5 and replace with 2 ml fresh DC DIFFERENTIATION medium.

F. At day 7 DCs are ready to use. Pipette gently to dislodge cells from plate and wash 2 X in PBS to remove GM-CSF and IL-4. DCs are usually cultured an additional 2-24 hrs in base GMP DC medium (without GM-CSF or IL-4) before initiation of functional experiments. At this point it is fine to plate on either low adherence plastic if you want to recover cells for flow cytometry or regular tissue culture plastic if you wish the cells to be adherent (for example, for T cell stimulation, we would plate on 96-well cell cluster plastic to allow the DCs to adhere for various treatments and then T cells would be seeded on top of the DCs).

EXPECTED RESULTS

Image 4: monocyte production region within adherent EB after 10 days in complete factory medium [Note, the smooth round cells concentrated near the lower right are monocytes. When leaving these foci of monocyte generation and entering the surrounding medium, the monocytes are more vacuolated and often have small dendrites].

