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Microglia differentiation from human pluripotent stem cells

Forked from a private protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details about microglia differential from human pluripotent stem cells.

Attachments



[395-858.pdf](#)

104KB

Guidelines

Reference

A Highly Efficient Human Pluripotent Stem Cell Microglia Model Displays a Neuronal-Co-culture-Specific Expression Profile and Inflammatory Response.

Walther Haenseler, Stephen N. Sansom, Julian Buchrieser, Sarah E. Newey, Craig S. Moore, Francesca J. Nicholls, Satyan Chintawar, Christian Schnell, Jack P. Antel, Nicholas D. Allen, M. Zameel Cader, Richard Wade-Martins, William S. James, Sally A. Cowley.

Fully defined human pluripotent stem cell-derived microglia and tri-culture system model C3 production in Alzheimer's disease.

Sudha R. Guttikonda, Lisa Sikkema, Jason Tchieu, Nathalie Saurat, Ryan M. Walsh, Oliver Harschnitz, Gabriele Ciceri, Marjolein Sneeboer, Linas Mazutis, Manu Setty, Paul Zumbo, Doron Betel, Lot D. de Witte, Dana Pe'er and Lorenz Studer.

Materials

Reagents required

A	B	C
Reagents	Supplier	Cat no
BMP4	Miltneyi	130-111-165
SCF	Miltneyi	130-096-692
VEGF	Miltneyi	130-109-385
X-VIVO 15	Lonza	BE04-418
M-CSF	Miltneyi	130-096-492
IL-3	Miltneyi	130-095-069
Advanced DMEM/F12	Life Technologies	12634-010
N2 supplement	Life Technologies	17502-048
GlutaMAX	Life Technologies	35050-061
2-mercaptoethanol	Life Technologies	31350-010
Pen/Strep	Life Technologies	17502-048
IL-34	Miltneyi	130-108-977
GM-CSF	Miltneyi	130-093-864
Aggrewell 800 well (24 wells)	STEMCELL Technologies	34811
Anti-Adherence Rinsing Solution	STEMCELL Technologies	7010
6 well ULA plates	Corning®	CLS3471
Nunc™ EasYFlask™ Cell Culture Flasks	Thermo Fisher	156499
Strainers, 40 mm	Corning	352340
RPMI medium	Thermo Fisher	11875093
IMDM medium	Thermo Fisher	12440053
B-27 supplement minus vitamin A	Thermo Fisher	12587010
Accutase	Thermo Fisher	A1110501
D-PBS -Ca2+/-Mg2+ CTS	Thermo Fisher	A1285601

	A	B	C
	Y-27632 dihydrochloride	Miltenyi	130-106-538
	KnockOut™ Serum Replacement	Thermo Fisher	10828028

- ✕ Human BMP-4 **Miltenyi Biotec Catalog #130-111-165**
- ✕ Human SCF **Miltenyi Biotec Catalog #130-096-692**
- ✕ Human VEGF (165) IS **Miltenyi Biotec Catalog #130-109-385**
- ✕ Human M-CSF **Miltenyi Biotec Catalog #130-096-492**
- ✕ Human IL-3 **Miltenyi Biotec Catalog #130-095-069**
- ✕ Advanced DMEM/F-12 **Thermo Fisher Scientific Catalog #12634010**
- ✕ Gibco™ N-2 Supplement (100X) **Thermo Fisher Scientific Catalog #17502048**
- ✕ Glutamax **Gibco - Thermo Fisher Scientific Catalog #35050-061**
- ✕ Gibco™ 2-Mercaptoethanol (50 mM) **Thermo Fisher Scientific Catalog #31350010**
- ✕ Penicillin-Streptomycin **Gibco - Thermo Fisher Scientific Catalog #15140148**
- ✕ Human IL-34 **Miltenyi Biotec Catalog #130-108-977**
- ✕ Human GM-CSF **Miltenyi Biotec Catalog #130-093-864**
- ✕ AggreWell™800 24-well Plate, 1 pack 1 Plate **STEMCELL Technologies Inc. Catalog #34811**
- ✕ Anti-Adherence Rinsing Solution 100 mL **STEMCELL Technologies Inc. Catalog #7010**
- ✕ Corning® Costar® Ultra-Low Attachment Multiple Well Plate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #CLS3471**
- ✕ Nunc® EasYFlask® Cell Culture Flasks, T75, filter **Thermo Fisher Catalog #156499**
- ✕ Falcon 40 µm Cell Strainer **Corning Catalog #352340**
- ✕ RPMI 1640 Medium **Thermo Fisher Scientific Catalog #11875093**
- ✕ IMDM **Gibco - Thermo Fisher Scientific Catalog #12440053**
- ✕ B-27® Supplement (50X), minus vitamin A **Thermo Fisher Catalog #12587010**
- ✕ Accutase cell dissociation reagent **Gibco - Thermo Fisher Scientific Catalog #A1110501**
- ✕ Gibco™ CTS™ DPBS calcium magnesium **Thermo Fisher Scientific Catalog #A1285801** 1
- ✕ StemMACS™ Y27632 **Miltenyi Biotec Catalog #130-106-538**
- ✕ KnockOut® Serum Replacement **Thermo Fisher Catalog #10828028**
- ✕ APC/Fire™ 750 anti-human CD11b Antibody **BioLegend Catalog #301351**
- ✕ FITC Mouse Anti-Human CD45 Clone HI30 (RUO) **Becton Dickinson (BD) Catalog #560976**



⊗ Anti-Iba1 antibody **Abcam Catalog #ab5076**

⊗ PU.1 (9G7) Rabbit mAb **Cell Signaling Technology Catalog #2258S**

⊗ Anti-CD11b antibody [M1/70] **Abcam Catalog #ab8878**

⊗ Purified anti-human CD45 Antibody **BioLegend Catalog #304002**

⊗ Anti-TMEM119 antibody produced in rabbit **Merck MilliporeSigma (Sigma-Aldrich) Catalog # HPA051870**



Preparation of aggrewell 800 plates

- 1 Warm basal medium (DMEM-F12 with KOSR) and complete medium (Desired medium to plate cells).

- 2 Open AggreWell plates in a biosafety cabinet.

Note

NOTE: Do not expose AggreWell plates to organic solvents, including ethanol or isopropanol.

- 3 Pre-treat wells with Anti-Adherence Rinsing Solution as described below. Add Anti-Adherence Rinsing Solution to each well to be used, as follows:

	A	B
	24-well plate	500 µL
	6-well plate	2 mL

- 4 Centrifuge plate at  1300 x g, 00:05:00 in a swinging bucket rotor fitted with plate holders.

Note

NOTE: Plates must be well balanced. Prepare a balance plate using a standard plate filled with water to match the weight and position of the AggreWell plate.

- 5 Observe the plate under a microscope to ensure that bubbles have been removed from microwells. If bubbles remain trapped in any microwells, centrifuge at

 1300 x g, 00:05:00 . Aspirate Anti-Adherence Rinsing Solution from the wells.

- 6 Rinse each well with warm basal medium, as follows:



5m



5m



6.1

	A	B
	24-well plate	2 mL

6.2 Aspirate medium from the well.

7 Add warm complete medium to each well to be used, as follows:



	A	B
	24-well plate	1 mL

Generation of EBs

8 Prepare a single-cell suspension in the desired medium.

9 To achieve EBs size of 10,000 cells per EB, plate 3.0×10^6 in each well and add complete medium to each well to achieve a final volume as follows:

	A	B
	24-well plate	2 mL/well

10 Prepare a centrifuge balance plate using a standard plate filled with water to match the weight and position of the AggreWell plate.

11 Pipette cells up and down gently several times to ensure even distribution of cells throughout the well.

Note

Be careful not to introduce bubbles into the microwells.



12 Immediately centrifuge the AggreWell plate at  100 x g, 00:03:00 to capture cells in the microwells, using the balance plate prepared.

3m



13 Observe the plate under a microscope to verify that cells are evenly distributed among the microwells.



14 Incubate the plate at  37 °C with 5% CO₂ and 95% humidity for  24:00:00 . Observe the cells under a microscope.

1d



Changing medium in aggrewell plate

15 Warm complete medium.

16 Perform a 50-75% medium change as follows: 24-well plate: Slowly remove 1-1.5 mL of medium from each well.

17 Replace with 1-1.5 mL of the fresh complete medium by slowly pipetting down the wall of the well. Slowly dispensing the medium helps to prevent displacement of EBs/spheroids from the microwells.

Harvesting EBs from aggrewell plates

18 Warm basal medium and complete medium.

19 Using a serological pipette:

Note

Alternative option: This step can be achieved by using P1000 cut tips.

19.1 Remove approximately half of the culture medium from the well.

19.2 Dispense the medium firmly back onto the surface of the plate to dislodge the EBs/spheroids from the microwells. Do not triturate.



- 20 Select the appropriate strainer and conical tube for separation of EBs/spheroids from single cells: 24-well plate, For harvesting from a single well, use 37- μ m Reversible Strainer, Small and a 15-mL conical tube.
- 21 Place strainer on top of the tube with the arrow pointed upward. Add  1 mL of PBS to wet the surface of the strainer.

- 22 Gently aspirate the dislodged EBs/spheroids. Pass the EB/spheroid suspension through the strainer.

Note

NOTE: The aggregates will remain on the filter; any unincorporated single cells will flow through.

- 23 Using a serological pipette, dispense  1 mL (24-well plate) or  3 mL (6-well plate) of the warm basal medium across the entire surface of the well to dislodge any remaining EBs/spheroids. Collect wash and pass over the strainer. 
- 23.1 Dispense  1 mL (24-well plate) or  3 mL (6-well plate) of the warm basal medium across the entire surface of the well to dislodge any remaining EBs/spheroids. (1/3)
- 23.2 Dispense  1 mL (24-well plate) or  3 mL (6-well plate) of the warm basal medium across the entire surface of the well to dislodge any remaining EBs/spheroids. (2/3)
- 23.3 Dispense  1 mL (24-well plate) or  3 mL (6-well plate) of the warm basal medium across the entire surface of the well to dislodge any remaining EBs/spheroids. (3/3)
- 23.4 Wash can be repeated until you do not see more EBs under the microscope.
- 24 Invert the strainer, and place over a new conical tube of the same size. Collect the EBs/spheroids by washing with 2-5 mL of complete medium per well harvested. 

Differentiation protocol

- 25 Maintain the pluripotent hPSCs/IPSCs on Lam-521 ($0.5 \mu\text{g}/\text{cm}^2$) in iPS-Brew medium. Passage cells can be every 7 days with $[1\text{M}]$ 0.5 millimolar (mM) EDTA, followed by seeding at a density of 2,500 cells per cm^2 with ROCK inhibitor ($[1\text{M}]$ 10 micromolar (μM) Y-27632) included in the medium for the first  24:00:00 after plating.
- 26 **Day 1:** Seed 3×10^6 iPSCs or ES cells into an Aggrewell 800 well (STEMCELL Technologies) to form EBs with ROCK inhibitor ($[1\text{M}]$ 10 micromolar (μM) Y-27632), in iPS-Brew medium and feed medium daily with BMP4 ( 50 μL), VEGF ( 50 μL), SCF ( 20 μL).
- 27 **Day 2- 4:** Change medium gently in the Aggrewell 800 well plate without agitating the EBs on the plate.
- 28 **Day 4:** Differentiate four-day EBs in either 6-well plates (15 EBs/well), T75 (75 EBs), or T175 flasks (150 EBs) with X-VIVO15 and supplement with  100 μL M-CSF,  25 μL IL-3, $[1\text{M}]$ 2 millimolar (mM) GlutaMAX,  100 μL penicillin,  100 μL streptomycin, $[1\text{M}]$ 0.055 millimolar (mM) b-mercaptoethanol with fresh medium added weekly.

Note

Maintain cells in this medium for up to 6 months.

Note

Note: At this point, pMacpre can be harvested and frozen down for further use.



- 30 Collect cells in suspension and either (a) Culture in RPMI with 10% FBS, l-glutamine, and pen/strep with IL-34 ( 100 μL) and M-CSF ( 10 μL) for 7–11 d until cells were adherent and elongated to transition to primitive macrophages, or (b) Co-culture with DA neurons in Neurobasal medium containing B-27 supplement (1:500), l-glutamine (1:1000) and BDNF ( 20 μL), ascorbic acid ( 0.2 millimolar (mM)) GDNF ( 20 μL), cAMP ( 500 micromolar (μM)), and IL-34 ( 100 μL) and M-CSF ( 20 μL) for 7–10 days for direct transition to microglia.
- 31 For serum-free differentiation, harvest and culture cells in suspension in 75% IMDM, 25% RPMI medium containing B-27 supplement (1:500), l-glutamine (1:1000) and IL-34 ( 100 μL) and M-CSF ( 20 μL) for 11–14 days.

Quality control for pMacpre and microglia

- 32 FAC-sorting for CD11b+ and CD45+ on both pMacpre and Microglia.

	A	B	C
	Antibodies	Supplier	Cat no
	CD11b	Biolegend	301351
	CD45 BD	Pharmin gen	560976

- 33 ICC for IBA-1, PU.1, TMEM119 on differentiated Microglia.

	A	B	C
	Antibodies	Supplier	Cat no
	IBA-1	AbCam	ab5076
	PU.1	Cell Signaling	2258S
	CD11b	AbCam	ab8878
	CD45	BioLegend	304002
	TMEM119	Sigma	HPA051870

