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ab317488 ioMicroglia – Human iPSC-Derived Microglia

View ab317488 ioMicroglia - Human iPSC-Derived microglia datasheet:

http://www.abcam.com/ab317488

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For conversion into consistent, mature, functional microglia providing a high quality human model for the study of neurological activity and disease.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

ioMicroglia rapidly mature into functional microglia after revival in the recommended medium. They are delivered in a convenient cryopreserved format and provide a homogeneous and reproducible model for human microglia.

ioMicroglia cultures consist mainly of microglia (>80%) characterised by the expression of TMEM119 and P2RY12, key microglia markers, & the IBA1 macrophage marker. These cells display expected cytokine secretion of IL-6, IL-8, IL10, IL-12p70, IL-1β & TNF-α (upon stimulation) and a high percentage of cells display desired microglia phagocytic function.

The protocol for the generation of ioMicroglia is a three-phase process. Phase 0 - Induction is carried out before distribution (Fig 1).

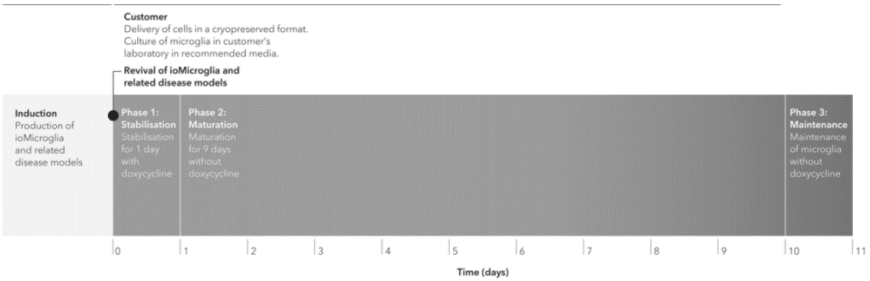


Figure 1 Schematic representation of the three-phase protocol to generate and culture ioMicroglia.

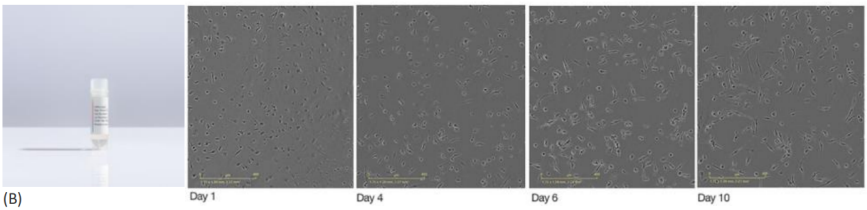
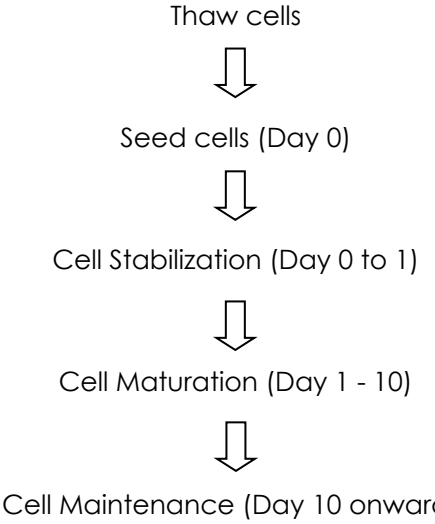


Figure 2 Photos of microglia after revival over the course of the first 10 days of culture (Day 1 to 10 post-thawing; 100X magnification; scale bar: 400µm).

Manufacturer	**Phase 0 — Induction (day -3 to 0):** Human iPSCs are exposed to a 3-day induction protocol. This leads to irreversible loss of pluripotency, and the synchronised homogenous production of sensory neurons. The ioMicroglia are subsequently cryopreserved for distribution.
User	**Phase1 — Stabilization (day 0 to 1):** The ioMicroglia are revived at the user's laboratory using the recommended medium supplemented with doxycycline (96h) for sustained induction.
	Phase 2 — Maturation (day 1 to 10): the ioMicroglia require full media change switching to a maturation medium with the removal of doxycycline & ROCK inhibitor
	Phase 3 — Maintenance (day 10 - onwards): Depending on assay requirements, the ioMicroglia can be used over different lengths of time in the maintenance medium. Note: ioMicroglia have been maintained up to 14 days in the above conditions without impairment to neuronal health, function and culture attachment

Table 1 Description of the three-phase protocol for the production and culture of ioMicroglia.

2. Protocol Summary



3. Materials Supplied and Storage

Transfer the vials of ioMicroglia to liquid nitrogen or to -150°C immediately after receipt.

The recommended reagents for the revival and maintenance of ioMicroglia can be found in Section 7.

4. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully culture the cells:

- Biological Safety Cabinet
- Normoxic cell culture incubator (37°C, 5% CO_2)
- 37°C water bath or equivalent
- Haemocytometer or automatic cell counter
- Liquid Nitrogen Storage Unit
- Standard tissue culture wares (pipettes, tips, culture plates)
- Bench Top Centrifuge
- Poly-L-Lysine Solution (0.01%) PDL-hydrobromide
- Sterile water
- Advanced DMEM/F-12
- B27
- N-2
- Glutamax
- 2-Mercaptoethanol (50 mM)
- M-CSF
- IL-34
- Rocki (ab144494)
- Doxycycline (ab141091)
- Bovine Serum Albumin

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For typical data produced using the cells, please see the ioMicroglia datasheet on our website.

6. Reagent Preparation

6.1 Preparation of stock solutions

Reagent	Stock Solution	Working concentration
M-CSF	10 µg/mL To prepare, reconstitute 50 µg in 5 mL of PBS containing 0.1% BSA	For comp:MS+D - 50 ng/mL 5 µL of stock solution per 1 mL of medium For comp:MM – 10 ng/mL 1 µL of stock solution per 1 mL of medium For comp:MM2x – 20 ng/mL 2 µL of stock solution per 1 mL of medium Working conc. for comp:MM2x dilutes to 10 ng/mL after the half media change
IL-34	10 µg /mL To prepare, reconstitute 50 µg in 5 mL of PBS containing 0.1% BSA	For comp:MM – 100 ng/mL 10 µL of stock solution per 1 mL of medium For comp:MM2x – 200 ng/mL 20 µL of stock solution per 1 mL of medium Working conc. for comp:MM2x dilutes to 100 ng/mL after the half media change

ROCK Inhibitor	10 mM (1,000X solution) To prepare, reconstitute 10 mM in 2 mL of sterile water	10 µM 1 µL of stock solution per 1 mL of medium
Doxycycline (DOX) (ab141091)	0.2 mg/mL (2,000X solution) To prepare, reconstitute 20 mg in 100 mL of H_2O	0.1 µg/mL 0.5 µL of stock solution per 1 mL of medium

6.2 Preparation of Microglia Medium

b:M: Microglia Basal Medium

Reagent/Media	For 200 mL	For 500 mL
Advanced DMEM F12	195.8 mL	489.5 mL
Glutamax (100x)	2 mL	5 mL
N2 (100x)	2 mL	5 mL
2-Mercaptoethanol (final conc. 50 µM)	200 µL	500 µL

Δ Note: The basal medium is stable for 3 weeks at 4°C; Pen/Strep antibiotics can be added if required.

comp:MS+D: Complete Microglia Stabilisation Medium with Doxycycline

Reagent/Media	For 50 mL	For 200 mL
b:M	49 mL	196 mL
B27 (50X)	1 mL	4 mL
M-CSF	250 µL	1000 µL
Doxycycline (final conc. 1 µg/mL)	25 µL	100 µL
ROCK Inhibitor	50 µL	200 µL

Δ Note: The complete medium is better prepared fresh before each feeding.

comp:MM: Complete Microglia Maturation Medium

Reagent/Media	For 10 mL	For 50 mL
b:M	10 mL	50 mL
IL-34	100 µL	500 µL
M-CSF	10 µL	50 µL

Comp:MM2x: Complete Microglia Maturation & Maintenance Medium

Reagent/Media	For 10 mL	For 50 mL
b:M	10 mL	50 mL
IL-34	200 µL	1000 µL
M-CSF	20 µL	100 µL

Δ Note: The supplemented complete medium is better prepared fresh before each feeding. It is not recommended to use the supplemented complete medium for more than 3 days after preparation while stored at 4°C.

6.3 Preparation of the Poly-L-Lysine coated vessels

Recommend use of pre-made Poly-L-lysine for ease of use of protocol. However, details on how to make Poly-L-Lysine solution from lyophilised powder is detailed below:

6.3 Preparation of Poly-L-Lysine coating solution from lyophilised powder:

6.3.1.1 Make up 50 mL of sterile water.

6.3.1.2 Resuspend a 5 mg vial of Poly-L-Lysine in 50 mL of the sterile water, for a working concentration of 100 µg/mL

6.3.2 Poly-L-Lysinecoating:

6.3.2.1 Calculate the total surface area to be coated.

6.3.2.2 Coat the surface area of your culture vessel with the Poly-L-Lysine coating solution. Recommended coating volumes are shown in the table below

6.3.2.3 Incubate the coated plates, at room temperature for 8 minutes.

6.3.2.4 Aspirate Poly-L-Lysine solution and then wash 3 times with sterile water. For each wash, use the same volume used for coating.

6.3.2.5 Asiprate the water and allow coated surfaces to dry completely in a laminar flow hood (without lids). This typically requires 120 minutes. Once dry, the plates are ready for use.

Coating	96 well	48 well	24 well	12 well	6 well
Poly-L-Lysine	50 µL	125 µL	250 µL	500 µL	1.25 mL

7. Assay Procedure

7.1 Culture of Microglia:

7.1.1 Before starting:

- Each ioMicroglia vial contains either ≥1.5 x10^6 6 (small vial) or ≥5 x 106 (large vial) viable cells. A seeding density of 39,500 cells/cm^2 is recommended. Prepare enough tissue culture vessels with Poly-L-Lysine coating prior to thawing the cryovial(s) (see Section 6.3).
- Warm-up the water bath to 37°C.
  Allow microglia basal medium (b:M medium) to reach room temperature prior to thawing of cells
- Prepare and warm up the complete: microglia stabilisation medium (comp:MS+D medium) to 37°C for revival (see Section 6.2).

7.1.2 Cell Thawing:

7.1.2.1 Remove the cryovial(s) from dry ice and immediately immerse into a 37°C waterbath (or similar) while maintaining a constant gentle agitation.

7.1.2.2 Remove the cryovial(s) from the water bath when only a very small ice cube is left visible (this should take approximately 2 minutes).

7.1.2.3 Spray the cryovial(s) with 70% ethanol and take it to a biological safety cabinet.

7.1.2.4 Transfer the cells from each vial into a 15 mL tube containing 1 mL of b:M medium.

Δ Note: Freezing medium contains DMSO: minimize the time between thawing and centrifugation of cells.

7.1.2.5 Transfer the cells from each vial into a single empty tube of the appropriate size (15 or 50 mL tube).

7.1.2.6 Carefully wash each cryovial with 1 mL of b:M medium and add it to the tube in a dropwise manner, agitating the tube occasionally ensuring the cell suspension is mixed with b:M medium before centrifugation.

7.1.2.7 Add 4 mL of b:M medium per tube in a dropwise manner.

7.1.2.8 Centrifuge the cells at 330 x *g* for 5 minutes at room temperature.

7.1.2.9 Return cells to biological safety cabinet and carefully remove the supernatant, without disturbing the cell pellet, by tilting the tube to a 45-degree angle and aspirating all of the medium from the meniscus up to the point where the tube starts to taper down towards the conical base.

7.1.2.10 Add 1 mL of comp:MS+D medium (with doxycycline) to the cell pellet and gently resuspend the cells by pipetting up-and-down with a 1 mL micropipette.

7.1.2.11 Count the cells including a cell viability marker. The typical recovery from one cryovial is ≥1.5 x10^6 viable cells.

7.1.3 **Cell Seeding (Day 0)**:

7.1.3.1 Dilute the cell suspension to the required cell concentration using comp:MS+D medium (with doxycycline) to achieve the required seeding density for your desired experimental conditions. A seeding density of 39,500 cells/cm^2 is routinely used.

Plate format	Surface (cm^2)	mL/well	Cells/well
6 well	9.5	2.5	375,000
12 well	3.8	1.0	150,100
24 well	1.9	0.5	75,050
48 well	0.95	0.25	37,525
96 well	0.32	0.2	12,640

7.1.3.2 Aspirate the coating solution from the culture vessel(s).

7.1.3.3 Directly add the required volume of cell suspension to the culture vessel(s).

7.1.3.4 Immediately transfer the culture vessel(s) to a standard normoxic tissue culture humidified incubator at 37°C, 5% CO_2.

7.1.3.5 To ensure an even cell distribution, gently cross-shake the plate once on the incubator shelf (back and forth, side to side, 2-3 times).

7.1.4 **Cell Stabilization (Day 0 to 1):**

7.1.4.1 Day 1: 24 hours post thawing, gently aspirate 90% of the media and replace it with fresh pre-warmed complete microglia maturation medium (comp:MM medium) (no doxycycline).

Δ Note: Culture of ioMicroglia should be carried out with special care as microglia are prone to mechanical stress which may cause detachment. It is recommended that for all medium replacements, medium aspiration and addition should be performed slowly and on the side of the well, using micropipettes instead of serological pipettes.

7.1.5 Cell Maturation and Maintenance (day 1 onwards):

7.1.5.1 For optimal microglia maturation and maintenance, a half-medium change every 72h for the remainder of the culture, replacing 50% of the medium with fresh complete microglia maturation & maintenance medium (comp:MM2x medium) (no doxycycline) is recommended.

8. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)