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ab317487 ioOligodendrocyte-like cells – Human iPSC-Derived ioOligodendrocyte-like cells

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1. Overview

ioOligodendrocyte-like cells are delivered in a convenient cryopreserved format and are programmed to rapidly mature upon revival in the recommended media.

ioOligodendrocyte-like cells are ready for experimentation upon thawing (day 1) and express the oligodendroglial lineage marker O4. By simply maintaining cells in a monolayer culture, cells rapidly mature and acquire a typical oligodendrocyte-like morphology with multiple branched processes. Cells start expressing oligodendrocyte markers, including MBP, PLP1, CNP and MAG.

The protocol for the generation of ioOligodendrocyte-like cells is a two-phase process. Phase 0 - Induction is carried out before distribution (Fig 1).

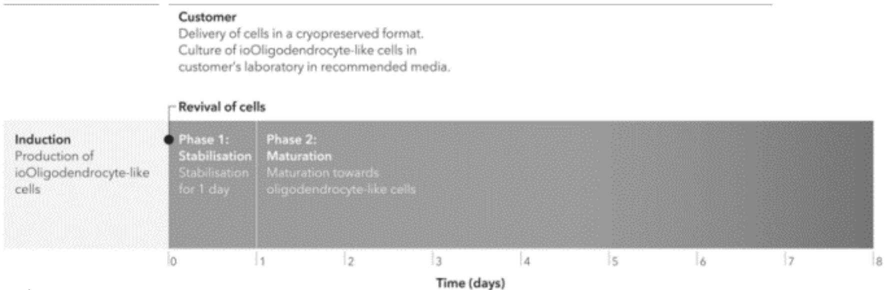

Figure 1 Schematic representation of the two-phase protocol to generate and culture ioOligodendrocyte-like cells.

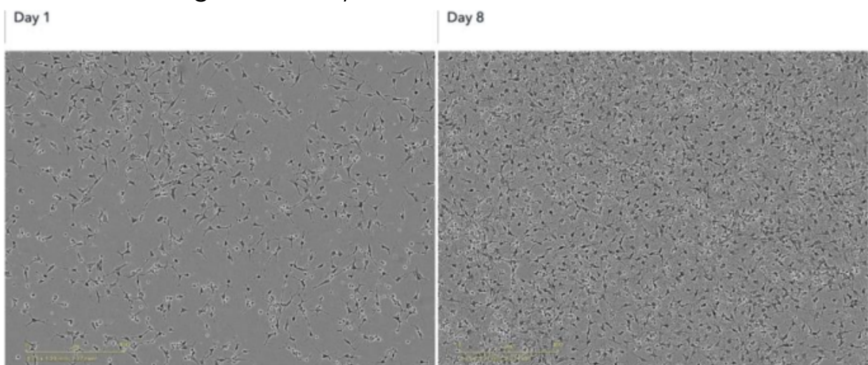

Figure 2 ioOligodendrocyte-like cells at day 1 and 8. Cells show rapid morphological changes, acquiring an oligodendrocyte progenitor cells (OPC)-like morphology by day 1 post-revival. By day 8, cells have matured and display an oligodendrocyte-like morphology (100X magnification; scale bar: 400µm).

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Manufacture r	**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 ioSensory Neurons 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 8): The ioOligodendrocyte-like cells require full media change switching to a maturation medium.

Table 1 Description of the two-phase protocol for the production and culture of ioOligodendrocyte-like cells.

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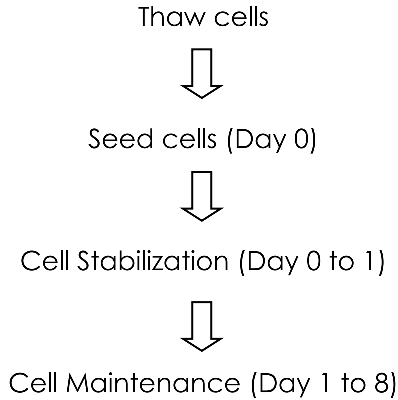
2. Protocol Summary



3. Materials Supplied and Storage

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

The recommended reagents for the revival and maintenance of ioOligodendrocyte-like cells can be found in Section 7.

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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-D-Lysine Solution
- Sterile water
- Laminin
- B27 (50X)
- N-2 (100X)
- Glutamax (100X)
- -mercaptoethanol (50 mM) M-CSF
- DPBS, no calcium, no magnesium
- Insulin
- T3
- Biotin
- cAMP
- NEAA (100X)
- PDGF-AA
- Animal-Free Recombinant Human FGF-basic
- Retinoic Acid
- Purmorphamine
- ROCK inhibitor
- NT-3
- IGF-I LR3
- Doxycycline (ab141091)
- NaOH
- BSA
- DMSO

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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 ioOligodendrocyte-like cells datasheet on our website.

6. Reagent Preparation

6.1 Preparation of stock solutions

Reagent	Stock Solution	Working concentration
T3	600 µg/mL To prepare, weigh 1.2 mg and dissolve in 2 mL of 1 N NaOH.	60 ng/mL 0.1 µL of stock solution per 1mL of medium.
Biotin	10 mg/mL To prepare, reconstitute 100 mg in 10 mL of 1 N NaOH. Make 25 µL aliquots and store them at -80°C. 1 mg/mL Dilute 25 µL of the 10 mg/mL stock in 250 µL of DMEM/F12 to make working aliquots. Keep aliquots at 4°C for up to 1 week.	100 ng/mL 0.1 µL of 1 mg/mL stock solution per 1 mL of medium
cAMP	10 mM To prepare, dissolve 5 mg in 1.017 mL of sterile water.	1 µM 0.1 µL of stock solution per 1 mL of medium

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Doxycycline (DOX) (ab141091)	2 mg/mL To prepare, reconstitute 20 mg in 10 mL of sterile water	1 µg/mL 0.5 µL of stock solution per 1 mL of medium.
PDGF-AA	100 µg/mL To prepare, reconstitute to 1 mg/mL in sterile water and dilute further to 100 µg/mL in PBS containing 0.1% BSA.	20 ng/mL 0.2 µL of stock solution per 1 mL of medium.
FGF-basic	4 µg/mL To prepare, reconstitute to 1 mg/mL in sterile water and dilute further to 4 µg/mL in PBS containing 0.1% BSA.	5 ng/mL 1.25 µL of stock solution per 1 mL of medium.
Retinoic Acid	100 mM To prepare, reconstitute 50 mg in 1.67 mL of DMSO. Store the stock at -80°C for up to 6 months. 1 mM Prepare working aliquots by diluting the stock solution in DMSO 100 times. Light sensitive, keep the aliquots away from direct light exposure.	100 nM 0.1 µL of 1 mM stock solution per 1 mL of medium.
Purmorphamine	10 mM To prepare, reconstitute 10 mg of powder in 1.92 mL of DMSO.	1 µM 0.1 µL of stock solution per 1 mL of medium.
ROCK inhibitor	10 mM To prepare, reconstitute vial in 2 mL of sterile water.	5 µM 0.5 µL of stock solution per 1 mL of

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		medium.
NT-3	50 µg/mL To prepare, reconstitute 25 µg in 500 µL of PBS containing 0.1% BSA.	5 ng/mL 0.1 µL of stock solution per 1 mL of medium.
IGF-I	100 µg/mL To prepare, reconstitute 1 mg in 1 mL of sterile water and dilute further to 100 µg/mL in PBS containing 0.1% BSA.	10 ng/mL 0.1 µL of stock solution per 1 mL of medium.

6.2 Preparation of ioOligodendrocyte-like cells medium

comp:OM1: Complete ioOligodendrocyte-like cells Stabilisation Medium

Reagent/Media	For 10 mL	For 100 mL
DMEM/F-12	9.46 mL	94.59 mL
B27	200 µL	2 mL
N2	100 µL	1 mL
Glutamax	100 µL	1 mL
NEAA	100 µL	1 mL
Insulin	7 µL	70 µL
β-Mercaptoethanol	10 µL	100 µL
T3	1 µL	10 µL
Biotin	1 µL	10 µL
cAMP	1 µL	10 µL
PDGF-AA	2 µL	20 µL
FGF-basic	12.5 µL	125 µL
Retinoic Acid	1 µL	10 µL
Purmorphamine	1 µL	10 µL

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Doxycycline	5 µL	50 µL
ROCK inhibitor	5 µL	50 µL

Δ **Note:** The complete medium is better prepared fresh before each feeding. Do not use the complete medium for more than 2 days after preparation.

comp:OM2: Complete ioOligodendrocyte-like Cells Maturation Medium

Reagent/Media	For 10 mL	For 100 mL
DMEM/F-12	9.47 mL	94.73 mL
B27	200 µL	2 mL
N2	100 µL	1 mL
Glutamax	100 µL	1 mL
NEAA	100 µL	1 mL
Insulin	7 µL	70 µL
β-Mercaptoethanol	10 µL	100 µL
T3	1 µL	10 µL
Biotin	1 µL	10 µL
cAMP	1 µL	10 µL
NT-3	1 µL	10 µL
IGF-I	1 µL	10 µL
Doxycycline	5 µL	50 µL

Δ **Note:** The complete medium is better prepared fresh before each feeding. Do not use the complete medium for more than 2 days after preparation.

6.3 Preparation of the Poly-D-Lysine coated vessel(s)

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

6.3 Preparation of Poly-D-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-D-Lysine in 50 mL of the sterile water, for a working concentration of 100 µg/mL.
6.3.1.3 Dilute Poly-D-Lysine solution 1:1 with sterile water for a working concentration of 50 µg/mL.

6.3.2 Poly-D-Lysine coating:

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-D-Lysine coating solution (50 µg/mL). Recommended coating volumes are shown in the table below.

Coating	96 well	24 well	12 well	6 well
Poly-D-Lysine	50 µL	250 µL	500 µL	1.25 mL

6.3.2.3 Incubate the coated plates, at 37°C for at least 3 hours.
Note: plates can be coated overnight at 37°C with Poly-D-Lysine.
6.3.2.4 Following incubation, aspirate Poly-D-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 60 minutes. Once dry, the plates are ready for use.

6.4 Preparation of the Laminin coating solution

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Recommend laminin vials contain 1 mg of laminin dissolved at a concentration of 1-2 mg/mL in 50 mM Tris-HCl, with 150 mM NaCl. The volume generally used is approximately 700-800 µL.

6.4 Preparation Laminin coating solution:

6.4.1.2 Measure the volume within the laminin vial and adjust the laminin solution to a total volume of 1 mL by adding DPBS. This will produce a stock of 1 mg/mL.

6.4.1.3 Add the final 1mL laminin solution into 49 mL of DPBS to make a final laminin coating solution of 20 µg/mL (the equivalent of 1 mg into 50 mL of DPBS), in order to dilute laminin to a final concentration of 20 µg/mL.

6.4.2 Laminin coating:

6.4.2.4 Calculate the total surface area to be coated.

6.4.2.5 Coat the surface area of your culture vessel(s) with the laminin coating solution (concentration 20 µg/mL) ensuring the entire surface of the well is coated. We recommend the coating volumes shown in the table below.

Coating	96 well	24 well	12 well	6 well
Laminin	50 µL	250 µL	500 µL	1.25 mL

6.4.2.6 Incubate the coated plates at 37°C for 2 hours.

6.4.2.7 Aspirate laminin coating solution from culture vessel(s) immediately before seeding cells.

Note: *Laminin coatings are prone to dessication and coating solution should only be aspirated immediately prior to cell seeding.*

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7. Assay Procedure

7.1 Culture of ioOligodendrocyte-like cells

7.1.1 Before starting:

7.1.1.2 Each ioOligodendrocyte-like cells vial contains ≥1.0 x 10^6 viable cells. A seeding density of 27,000 cells/cm^2 is recommended.

7.1.1.3 Prepare enough tissue culture vessels with Poly-D-Lysine and laminin coating prior to thawing the cryovial(s) (see Section 6.3).

7.1.1.4 Remove the vials from liquid nitrogen immediately before step 3.1, and keep at -80°C or in dry ice. Do not store the cells at -80°C or in dry ice long term

7.1.1.5 Warm-up the water bath to 37°C.

7.1.1.6 Allow DMEM/F12 to reach room temperature prior to thawing of cells.

7.1.1.7 Prepare and warm up the complete: stabilization medium (comp:OM1 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 or 50 mL tube.

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

7.1.2.5 Carefully wash each cryovial with 1 mL of DMEM/F12 medium, using a 1 mL micropipette, and add it to the tube in a dropwise manner, agitating the tube occasionally ensuring the cell suspension is mixed with DMEM/F12 medium before centrifugation.

7.1.2.6 Add 3 mL of DMEM/F12 medium to the 15 or 50 mL tube in a dropwise manner while agitating the tube.

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7.1.2.7 Centrifuge the cells at 200 x *g* for 4 minutes at room temperature.

7.1.2.8 Carefully remove the supernatant by aspiration.

7.1.2.9 Add 1 mL of comp:OM1 medium to the cell pellet and resuspend the cells by pipetting gently up-and-down with a 1 mL micropipette, 3 to 5 times.

7.1.2.10 Count the cells including a cell viability marker. The typical recovery from one cryovial is ≥1.0 $x10^6$ viable cells.

7.1.3 Cell Seeding (Day 0):

7.1.3.1 Dilute the cell suspension to the required cell concentration using comp:OM1 medium to achieve the required seeding density for your desired experimental conditions. A seeding density of 27,000 cells/cm^2 is routinely used.

Plate format	Surface (cm^2)	mL/well	Cells/well
6 well	9.6	2.5	259,200
12 well	3.8	1.0	102,600
24 well	1.9	0.5	51,300
96 well	0.32	0.2	8,640

7.1.3.2 Aspirate the laminin 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).

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7.1.4 Cell Stabilization (Day 0 to 1):

7.1.4.1 Day 1: 24 hours post thawing, completely replace the culture media with fresh pre-warmed complete maturation medium (comp:OM2 medium).

Δ **Note:** Culture of ioOligodendrocyte-like cells 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 (day 1 to 8):

7.1.5.1 Day 1 to day 8: for optimal ioOligodendrocyte-like cells culture, a half-medium change every 48 hours, replacing 50% of the medium with fresh complete maturation medium (comp:OM2 medium) is recommended

Δ **Note:** ioOligodendrocyte-like cells cultures are not recommended to use longer than day 8 post revival

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8. Notes

Technical Support

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