

AXOL

**Human iPSC-derived
retinal organoids**

User guide

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Receiving and maintaining retinal organoids

General Notes

- Organoids will be shipped in 5ml microcentrifuge tubes in their cell culture medium
- All work with live organoids should be performed in a Class II laminar flow tissue culture hood using aseptic technique
- Organoids are to be maintained in a humidified tissue culture incubator (37°C, 5% CO₂, 20% O₂)
- We recommend allowing at least 2 days for retinal organoids to acclimatise before assaying
- Cell culture medium needs to be stored at 4°C for up to 1 month and pre-warmed to room temperature prior to use

Materials and reagents provided by Axol Bioscience

- 3 ml sterile Pasteur pipettes (x3)
- 96-well plate for culturing organoids
- Organoid maintenance medium

Materials and Reagents provided by the user

- For all customers
 - Fetal Bovine Serum (FBS) (recommended Fisher-Scientific, product code: 10082147 or 15383681)
- For organoids day 120 or less
 - Retinoic acid (recommended Sigma-Aldrich, product code: R2625)

Packaging

- The organoids and the media are delivered in two separate QuickSTAT CREDO boxes with temperature monitor

DO NOT DISPOSE OF THE BOX OR THE MONITOR.

- QuickSTAT will organize pick up and return of the boxes and monitor.

Procedure

1. Preparation of organoid maintenance medium

a. Please add 10% (v/v) FBS to organoid maintenance medium upon receipt of the organoids.

For customers receiving less than 50 organoids, add 15ml FBS to 135ml maintenance medium. For customers receiving more than 50 organoids, add 30ml FBS to 270ml maintenance medium.

b. For customers receiving organoids day 120 or less: prepare and store aliquots of retinoic acid following the manufacturer's guidelines. Protect aliquots from light and do not re-freeze. For every feed, transfer the required volume of maintenance media to a sterile tube/bottle and supplement with retinoic acid at a final concentration of 0.5 μ M. Feed organoids with maintenance media supplemented with retinoic acid until they reach 120 days of differentiation. Organoids older than day 120 should be fed with maintenance media only.

2. Transfer each individual organoid to a well of the provided 96-well plate by following the procedure described below:

a. Using a Pasteur pipette, transfer each of the organoids into separate wells of the plate, one organoid per well **b.** Avoid direct contact of the Pasteur pipette with the organoids: allow the organoids to be carried within the medium **c.** Let the organoids settle with gravity (this should take ~ 1 minute)

3. Using a P200 pipette, remove as much medium as possible but ensure the organoids remain covered

Note: avoid touching the organoids as it may damage them

4. Add 200 μ L of fresh organoid maintenance medium to each well containing the organoids

5. Replenish cell culture medium by removing half of the medium and adding 100 μ L of fresh medium per well

a. For customers receiving organoids older than day 120: Cell culture medium should be replenished twice a week (e.g. Monday and Friday) **b. For customers receiving organoids day 120 or less:** cell culture medium should be replenished three times a week (e.g Monday, Wednesday and Friday) **Note:** for organoids that are day 120 or less, supplement maintenance medium with fresh retinoic acid (please see Step 1b).

Technical sheet

Specifications

Format	Cell types	Donor
10 retinal organoids per 5 ml microfuge tube 150 ml of optimized cell culture medium per 100 organoids 2 x 96 well plates per 100 organoids 2 x Pasteur pipettes	Cone and Rod photoreceptors Retinal ganglion cells (RGCs) Bipolar cells Horizontal cells Amacrine cells Müller glial cells	Human, male, 68-year-old

Readouts

- Immunofluorescence analyses
- Gene expression by RT-qPCR
- Transcriptomic analysis by single-cell RNA sequencing
- Cytotoxicity assays
- Cytokine release
- Flow cytometry
- Electron microscopy

Gene expression in retinal organoids through different stages

Cell Type	Gene	Timepoint of appearance	Timepoint of peak expression
Retinal ganglion cells	MATH5 (ATOH7)	d30-d180	d60
Retinal ganglion cells	BRN3 (POU4F2)	d30-d210	d60
Horizontal and amacrine cells	TFAP2A	d30-d210	d150
Horizontal and amacrine cells	PROX1	d30-d210	d150
Bipolar cells	GRIK1	d30-d210	d150
Bipolar cells	CADPS	d30-d210	d150
Photoreceptors	RP11VS	d60-d210	d210
Photoreceptors	RBP3	d60-d210	d210
Photoreceptors	IMPG1	d120-d210	d210
Photoreceptors	CRX	d60-d210	d210
Cone photoreceptors	OPN1SW	d120-d210	d210
Cone photoreceptors	OPN1MW	d150-d210	d210
Cone photoreceptors	OPN1LW	d120-d210	d210

Cone photoreceptors	ARR3	d60-d210	d180
Cone photoreceptors	RXRG	d60-d210	d150
Rod photoreceptors	RHO	d120-d210	d210
Rod photoreceptors	NRL	d90-d210	d180
RPE	RPE65	d60-d210	d210
Muller glia	RLBP1	d90-d210	d210
Muller glia	CRYM	d30-d210	d210

Expected protein expression in retinal organoids at d150 and d180

Cell Type	Cell marker	Protein expression at d150	Protein expression at d180	Protein localization at d150-d180
Photoreceptors	RCVRN	Yes	Yes	ONL
Retinal ganglion cells	SNCG and HuC/D	Yes	Yes	INL/GCL
Cone photoreceptors	OPN1MW/LW	*	Yes	ONL
Rod photoreceptors	RHO	*	Yes	ONL
Bipolar cells	PKC-a	**	Yes	INL
Amacrine cells	AP-2a	Yes	Yes	INL
Horizontal cells	PROX1	Yes	Yes	INL
Muller glia	CRALBP	Yes	Yes	All layers

* small number of developing rods and cones ** Expressed at transcriptional level

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