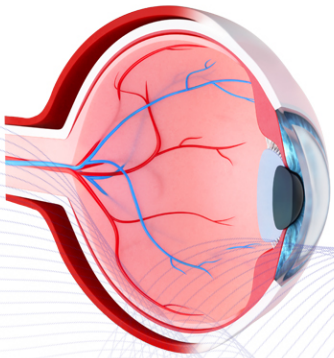
RETINAL ORGANOIDS



RETINA

An in vitro, light responsive retinal model
for accurate predictions of in vivo outcomes

The Retinal Organoids model from Newcells recapitulates the complex structure of the retina

Product Specification

Format	10 organoids per 5ml microfuge tube 150 ml of optimized cell culture medium per 100 organoids 2 x 96 well plates per 100 organoids 2 x Pasteur pipettes
Cell Types	Organoids Rod and Cone photoreceptors Retinal ganglion cells (RGCs) Bipolar cells Horizontal cells Amacrine cells Müller glial cells RPE (expected availability 2022) Retinal pigment epithelial cells
Species	Human Rat (In development) Non-human primate (In development)
Available analytical readouts	Immunofluorescence analyses Gene expression by RT-qPCR Transcriptomic analysis by single-cell RNA sequencing Cytotoxicity assays Cytokine release Flow cytometry Electron microscopy Custom assays
Origin	Heathy donor Patient samples
Assay Widow	≥30 days

- ✓ The organoids are ~1.3 mm in diameter and contain ~40,000 cells.
- ✓ Tested for different applications including gene therapy, toxicology and retinal disease modelling.
- ✓ They form primitive photoreceptor outer segments leading to responsiveness to light.
- ✓ They respond to known toxins similar to that seen in vivo.

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
	BRN3 (POU4F2)	d30 - d210	d60
Horizontal and amacrine cells	*TFAP2A*	d30 - d210	d150
	PROX1	d30 - d210	d150
Bipolar cells	*GRIK1*	d30 - d210	d150
	CADPS	d30 - d210	d150
Photoreceptors	*RCVRN*	d60 - d210	d210
	RBP3	d60 - d210	d210
	IMPG1	d120- d210	d210
	CRX	d60 - d210	d210
Cone photoreceptors	*OPN1SW*	d120 - d210	d210
	OPN1MW	d150 - d210	d210
	OPN1LW	d120 - d210	d210
	ARR3	d60 - d210	d180
	RXRG	d60 - d210	d150
Rod photoreceptors	*RHO*	d120 - d210	d210
	NRL	d90 - d210	d180
RPE	*RPE65*	d60 - d210	d210
Muller glia	*RLBP1*	d90 - d210	d210
	CRYM	d30 - d210	d210

Retinal organoid differentiation follows the developmental timeline of embryonic development of the retina with various cell types arising at different times in a sequential manner. Long-term cell survival is hauled by the limitations of the *in vitro* culture conditions and the limitations of the model itself. (e.g. absence of visual cortex).

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	✓	✓	ONL
Retinal ganglion cells	SNCG and HuC/D	✓	✓	INL/GCL
Cone photoreceptors	OPN1MW/LW	✓ *	✓	ONL
Rod photoreceptors	RHO	✓ *	✓	ONL
Bipolar cells	PKC-α	**	✓	INL
Amacrine cells	AP-2α	✓	✓	INL
Horizontal cells	PROX1	✓	✓	INL
Müller glia	CRALBP	✓	✓	All layers

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

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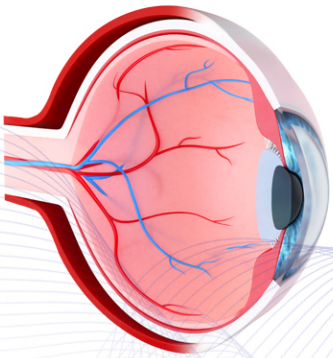
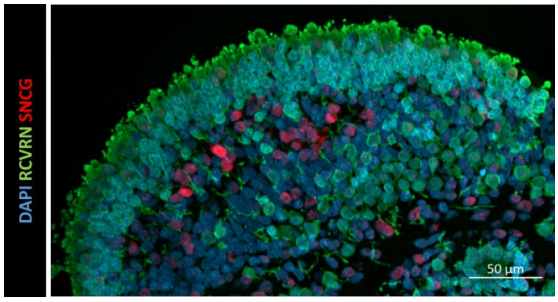
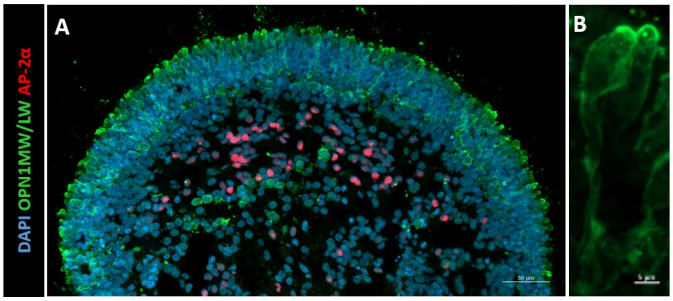


RETINA

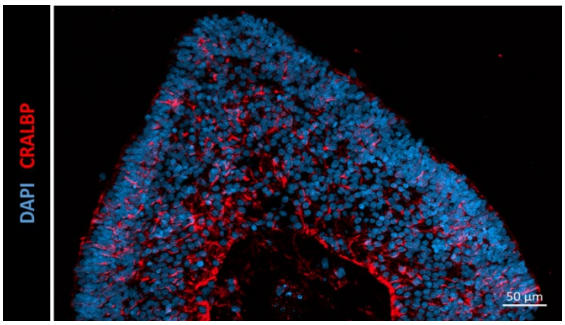
Characterization of iPSC-derived Retinal Organoids



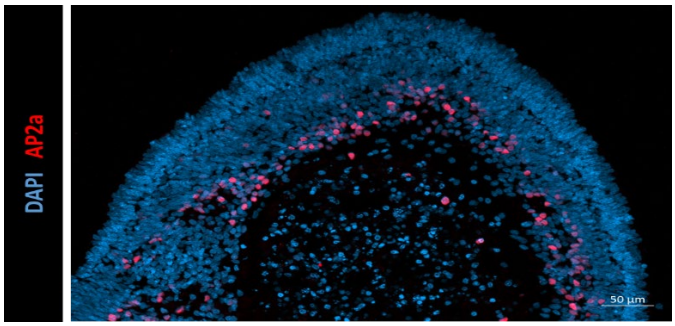
Localization and distribution of photoreceptors (RCVRN, green) and retinal ganglion cells (SNCG, red) in retinal organoids at d150.



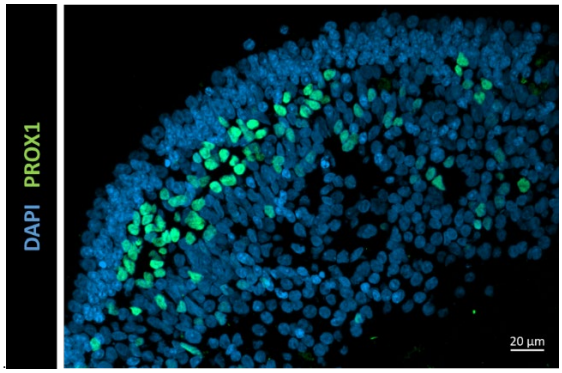
Representative IF image of retinal organoid at d210. (A) Localisation and distribution of cone photoreceptors (OPN1MW/LW, green) and amacrine cells (AP-2α, red); (B) detailed view of image A, showing cone photoreceptors with primitive outer segments.



Localization and distribution of Müller glia cells (CRALBP, red) in retinal organoids at d180.



Localization and distribution of amacrine cells (AP-2α, red) in retinal organoids at d150.



Localization and distribution of horizontal cells (PROX1, green) in retinal organoids at d150

Resources

Application of organoid technology for retinal disease modelling and drug discovery; Chichagova, Drug Target Review, June 2020
Application of organoid technology for retinal disease modelling and drug discovery (drugtargetreview.com)
Human iPSCs generate light responsive retinal organoids with variable and nutrient dependent efficiency; Hallam et al, Stem Cells, 2018, 36(10), 1535-1551
Human-Induced Pluripotent Stem Cells Generate Light Responsive Retinal Organoids with Variable and Nutrient-Dependent Efficiency - PubMed (nih.gov)
Room temperature shipment does not affect the biological activity of iPSC derived retinal organoids; Georgiou et al, PLOS One, 15(6), e0233860
Room temperature shipment does not affect the biological activity of pluripotent stem cell-derived retinal organoids (plos.org)
Enhancing immune function of hiPSC derived retinal organoids by incorporating microglial cells; Chichagova et al, Investigtive Ophthamology and VISUAL Science, 2020, 61(7)
Enhancing immune function of hiPSC-derived retinal organoids by incorporating microglial cells | IOVS | ARVO Journals
Human iPSC differentiation to retinal organoids in response to IGF1 and BMP4 activation is line and method dependent; Chichagova et al, Stem Cells, 2020, 38(2), 195-201
Human iPSC differentiation to retinal organoids in response to IGF1 and BMP4 activation is line- and method-dependent - PubMed (nih.gov)

The need for pre-clinical Retinal Organoids	Results with Newcells Retinal Organoids
Recapitulation of the complex architecture with the relevant cell types	A physiologically relevant, functional, light responsive model for mechanistic insights
Unlimited material for use in safety and efficacy studies	Simple pre-clinical studies for safety and efficacy
Lack of suitable models for the human retina for disease modelling for accurate pre-clinical data	Predictive disease modelling platform
Ethical responsibility related to the 3R principles (Replace, Reduce and Refine)	Reduction of use of animal models in line with NC3Rs and NA3RsC

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