amsbio

Organoid Culture *Handbook*

Reagents | Cells | Matrices

Accelerate Discovery Through Innovative Life Science

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Introduction to Organoid Culture

The concept of 3D cell culture has been around for over a century, when Wilson H. V. (1907) demonstrated that mechanically separated sponge cells were capable to differentiate and reorganize, growing into fully functional organisms. Nowadays 3D cell culture has gained a lot of attention and has become increasingly widespread since it can now be applied to mammalian cells. It is only with the recent advances of stem cell technologies and of mammalian developmental biology that 3D cell culture techniques have become wildly applicable. Organoids are cell-derived tissue and organ-like structures composed of one or more cell types that can be formed by 3D cell culture and differentiation of embryonic or induced pluripotent stem cells (ESCs and iPSCs), progenitor cells of particular organ of interest and also can be grown from a limited amount of starting material coming from tissue biopsies (adult stem cells, ASCs) (Figure 1). They are capable of recapitulating structures of tissues and organs and mimic their functions *in vitro*. While ESCs and ASCs are both of natural origin iPSCs are obtained by reprogramming of some specialized adult cells and remarkably are similar to ESCs. iPSCs have the potential to be used in patient specific treatments thus avoiding the risk of immune rejection and could potentially overcome ethical issues hampering the development of ESCs for clinical use. Research and therapeutic potential of organoids includes:

- ✓ Tissues morphogenesis & Organogenesis Models
- ✓ Tumor, Disease and Infection Models
- ✓ Drug Testing
- ✓ Toxicity Screening
- ✓ Personalized Medicine
- ✓ Regenerative Medicine / Organ Replacement

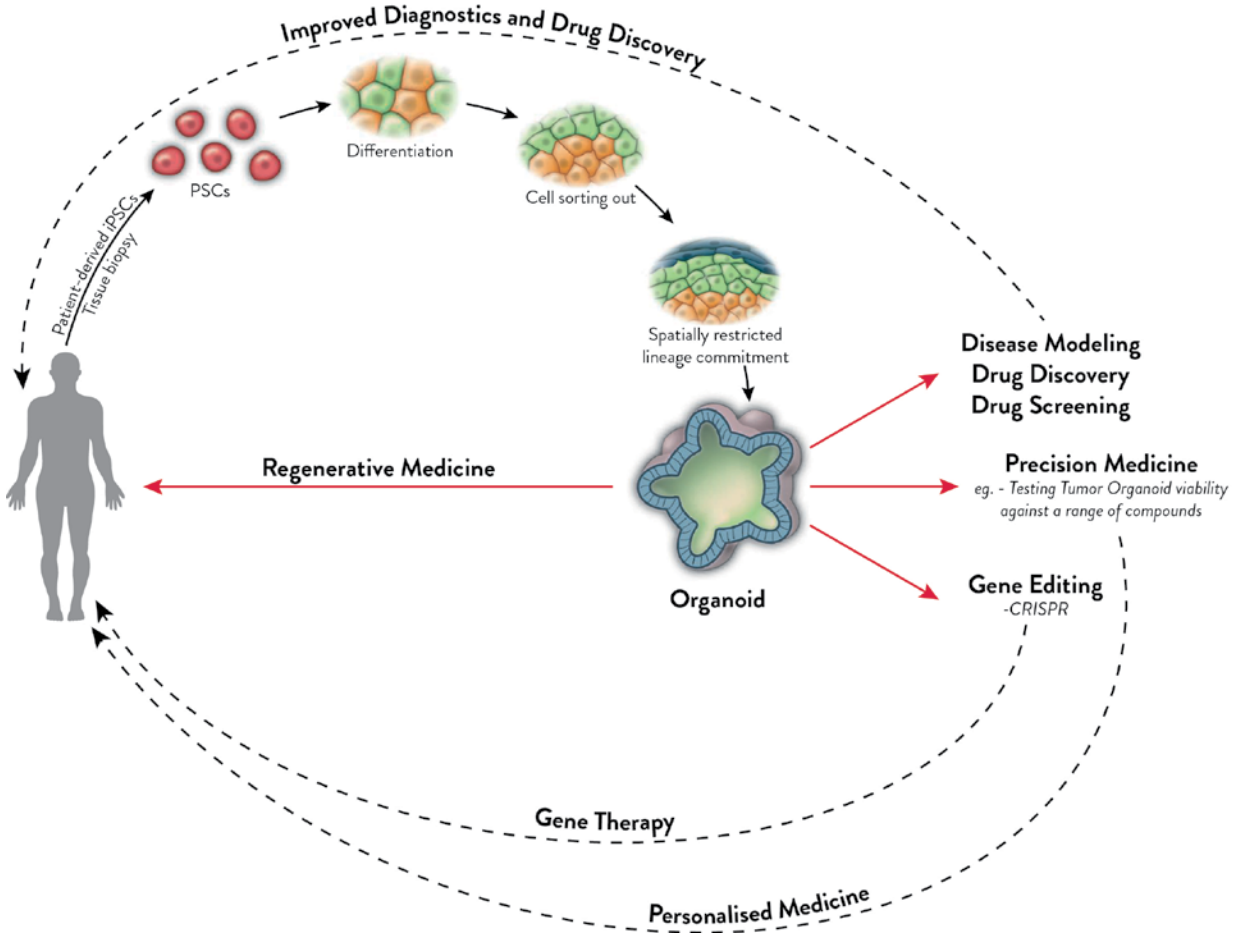


Figure 1. *Schematic representation of stages required in organoid formation and potential applications of organoids in different research fields leading eventually to extraordinary changes in people lifes quality . Organoids generated from iPSCs, created by reprogramming of specialized human adult cells, and ASCs coming from patients tissue biopsies can be used for improved diagnostics and to accelerate drug discovery, personalized medicine, gene therapy,regenerative medicine, and diseases research.*

Organoid culture is an advanced tool with tremendous potential to influence life sciences as currently used preclinical models (represented by 2D culture of cells and tissues or animals) are falling short in predicting biological responses. 2D culture is unable to maintain the natural physiological shape of the cells and therefore crucial cell-cell and cell-extracellular matrix interactions are lost. Tissues can be hard to obtain and there is high variability between samples. Animals which are usually rodents are not fully representative of the human body because have different physiological properties. All of these models fail to recreate the complexity and the specificity of living tissues and organs. Growing 'organs-in-a-dish' (ie. organoids) allows to overcome these challenges, making it possible to demonstrate the complexity of living tissues and organs *in vitro*.

While organoids are invaluable for research, their biggest potential lies in therapeutics, where we are already seeing their application in personalized medicine and oncology. The methodology for obtaining intestinal organoids developed by Hans Clevers is currently used in the Netherlands to screen cystic fibrosis patients for their compatibility with drugs currently available. At the Gurdon Institute in Cambridge, Meritxell Huch with co-workers are testing a variety of drugs on liver cancer organoids, highlighting compounds that successfully reduce or cause stagnation of tumour growth.

Current advances are already making an impact on improving the way we understand human body structuring as well as development of different processes and mechanisms occurring in human body and result in deepening our knowledge which can be employed at many fields. Diagnostics, personalized medicine, gene therapy and regenerative medicine are greatly influenced by the ongoing progress in organoids technology. Organoids enable the acceleration of drug discovery by employing high-throughput screening, where cell-derived *in vitro* 3D models of tissues and organs mimic the complex *in vivo* environments to an unparalleled level.

Cell Culture Feature/Effect	Cell Culture Type	
	2D	3D
Morphology	Shaped change (cells are flat with typical thickness of 3 μm) and polarization lost	Real natural shape (cells are ellipsoids with dimensions of 10-30 μm) and polarization conserved
Proliferation	Lower proliferation rate	More pronounced; experiments can be performed over a longer period of time
Differentiation	Non spontaneous	Can be spontaneous, caused by cellular contact or soluble factors
Migration & Invasion	Cell motility is reduced, cell direction is changed, very limited cell-ECM interaction	Very complex motility models taking into consideration not only stiffness but also the rheology and geometry of ECM
Angiogenesis	Only observational	Can be functional
Genetic profile	Modified	Preserved. Better representation of growth factors, pro-angiogenic and adhesion molecules genes
Multicellular studies	Better when studying immune response	Good in co-culture, but might be complicated with more than two cell types
In vivo relevance	Cannot serve as a model which truly represents tissues and organs	Recapitulate tissues and organs structure and enables to mimic their functions
Viability	Less resistant to treatment	More resistant to treatment
Resistance to treatment	Poor demonstration of drug uptake, penetration, effectiveness and its toxicity effects	More accurate reflection of drug uptake, penetration, effectiveness and its toxicity effects
Mathematical model	Possible but simplified	Better geometry, improves link between structure and function
Reproducibility	Short-term only	Quite high which might last for longer
Cost	From affordable to quite expensive depending on used techniques and equipment	Higher cost

Table 1. *Comparison of advantages and disadvantages of 2D and 3D cell cultures.*

Organoid Models

Organ	Images and Results
Brain	Pg 35
Retina	Pg 36
Esophagus	Pg 30, 31
Breast	Pg 32
Liver	Pg 26, 27, 28
Pancreas	Pg 33
Stomach	Pg 16
Small Intestine	Pg 28
Colon	Pg 29
Fallopian Tubes	Pg 34
Ovary	Pg34
Uterus (Endometrium)	Pg 34
Prostate	Pg 32, 33

Organoid Culture Protocols

Organoids can be grown from donor tissue, progenitor organ cells, embryonic stem cells, and induced pluripotent stem cells. This section describes how to generate and culture organoids from tissue and organ progenitor cells (p8-10) or stem cells (p11) based on peer-reviewed protocols.

These include the protocols established by the Hans Clevers Lab in 2009 and 2012 for growing intestinal organoids (p8-10) from intestinal adult stem cells [1-2] and the protocol for growing pancreas and liver organoids (p11) published by a group lead by Meritxell Huch at the Gurdon Institute [3] where extracellular matrix (ECM) was used. ECM enables formation of 3D cell cultures were cell-cell and cell-ECM interactions occur similarly as in live organisms.

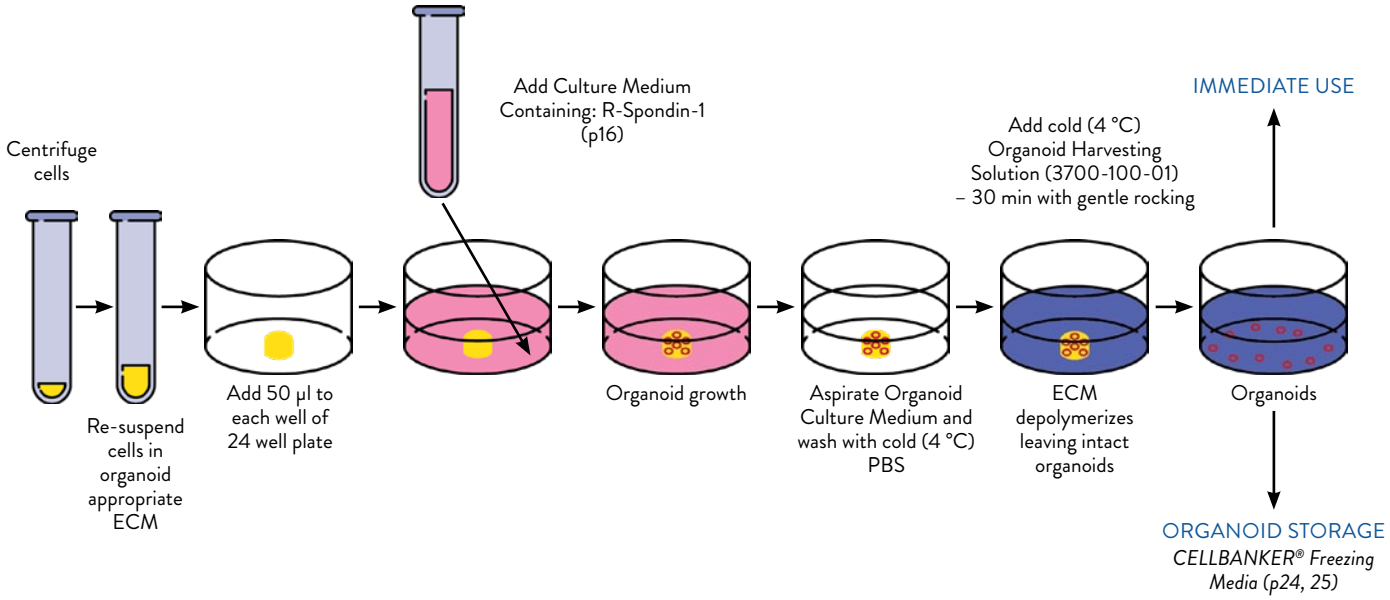
Calvin Kuo reported generation of intestinal organoids from tissue [4] by employing air-liquid interface method (p10).

In late 2016 Ryuji Morizane and Joseph V. Bonventre published a procedure detailing how to grow kidney organoids from human pluripotent stem cells[15]. This protocol recommends the use of StemFit® containing human albumin (p22), which facilitates maintenance and expansion of human stem cells ensuring reliable and well-defined growth condition, for passaging the pluripotent stem cells. A few months later Majlinda Lako and co-workers published a paper describing how they successfully made light-responsive retinal organoids from induced pluripotent stem cells (p11) [10]. Low-adhesion Lipidure®-COAT plates (p21) from AMSBIO were used to generate these organoids. New protocols for growing different kinds of organoids are emerging at an increasing rate. Please visit our website to find out about the most recent advancements in the field of organoids.

Contact us detailing what your research is about and we can recommend the best protocols and products needed to help you achieve your goals.

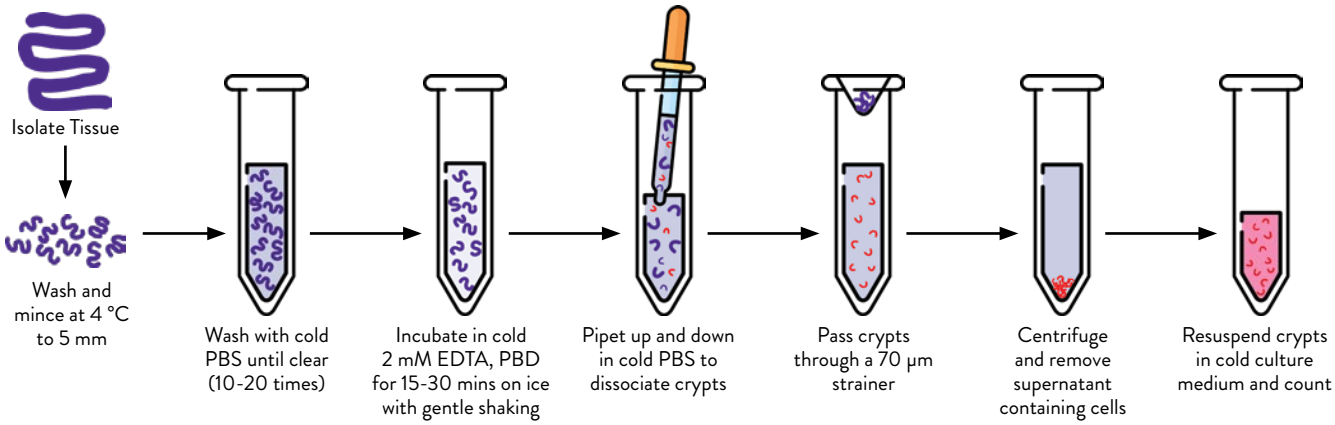
GENERAL SUBMERGED METHOD FOR ORGANOID CULTURE

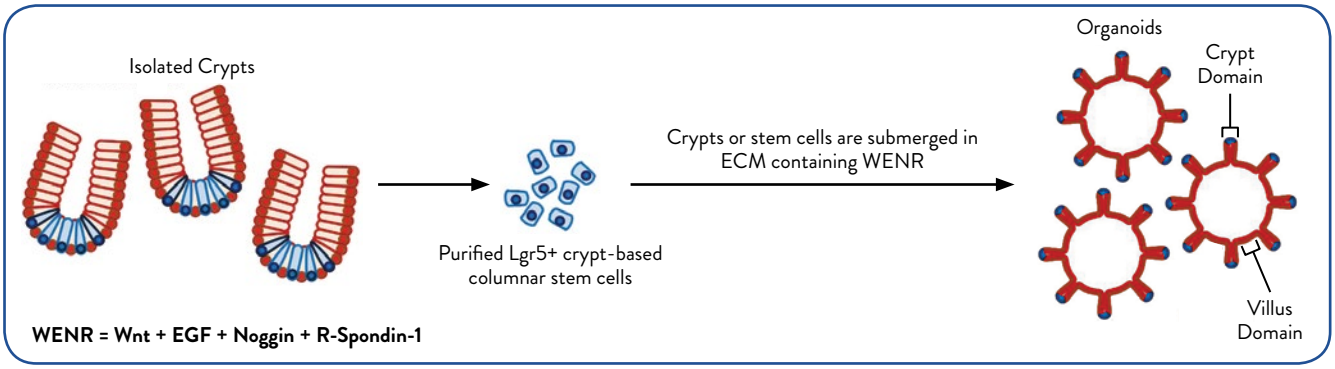
Developed by the Hans Clevers Lab, Hubrecht Institute, Netherlands



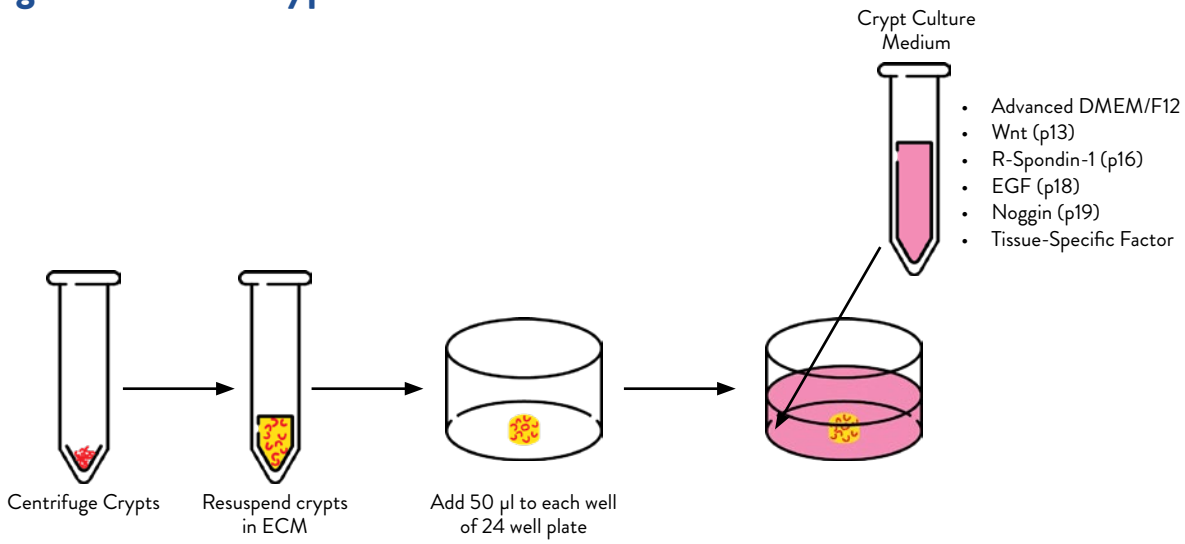
Crypt Isolation

Developed by the Hans Clevers Lab, Hubrecht Institute, Netherlands





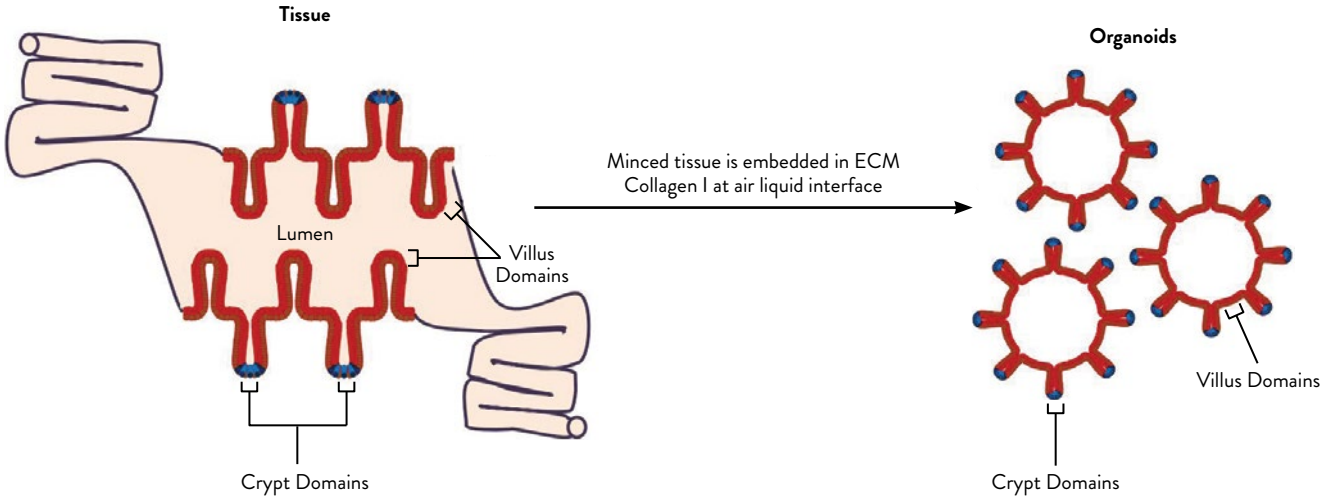
Organoids from Crypts



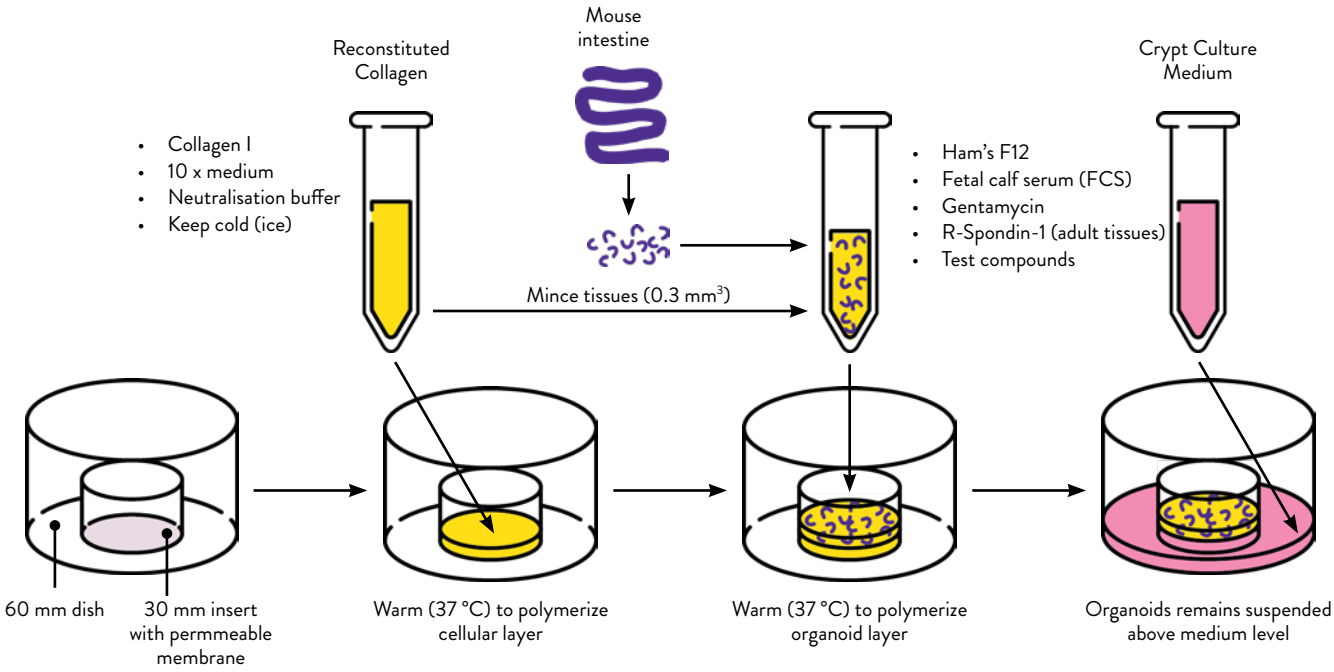
AIR LIQUID INTERFACE (ALI) METHOD FOR ORGANOID CULTURE

Developed by the Calvin Kuo Lab, Stanford University, USA

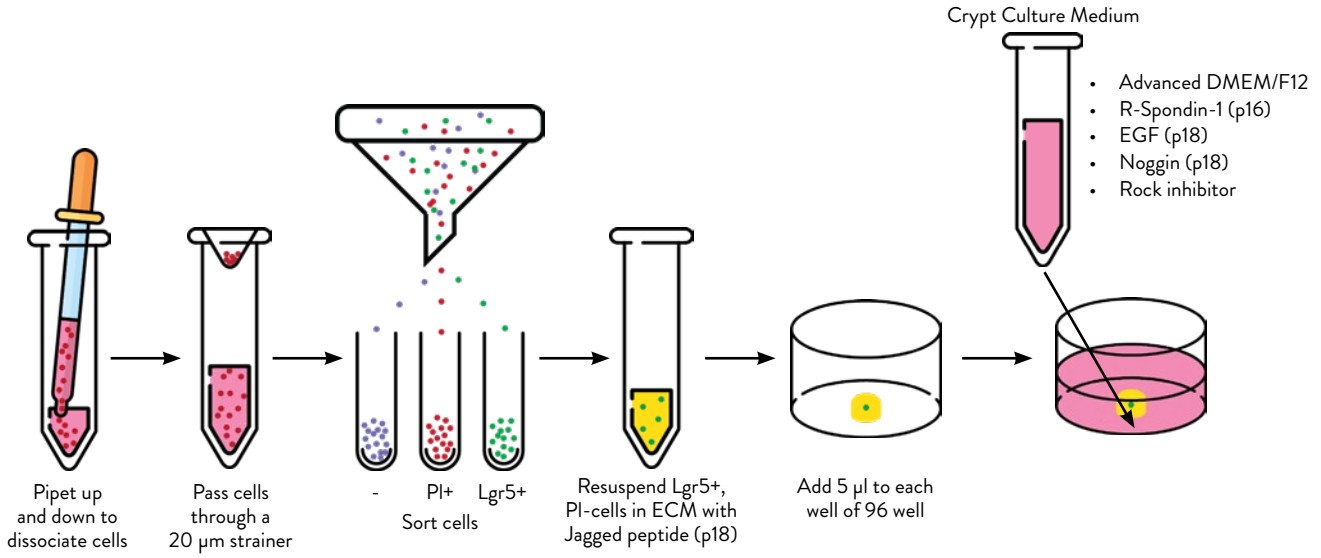
Organoids from Tissue



ALI Method



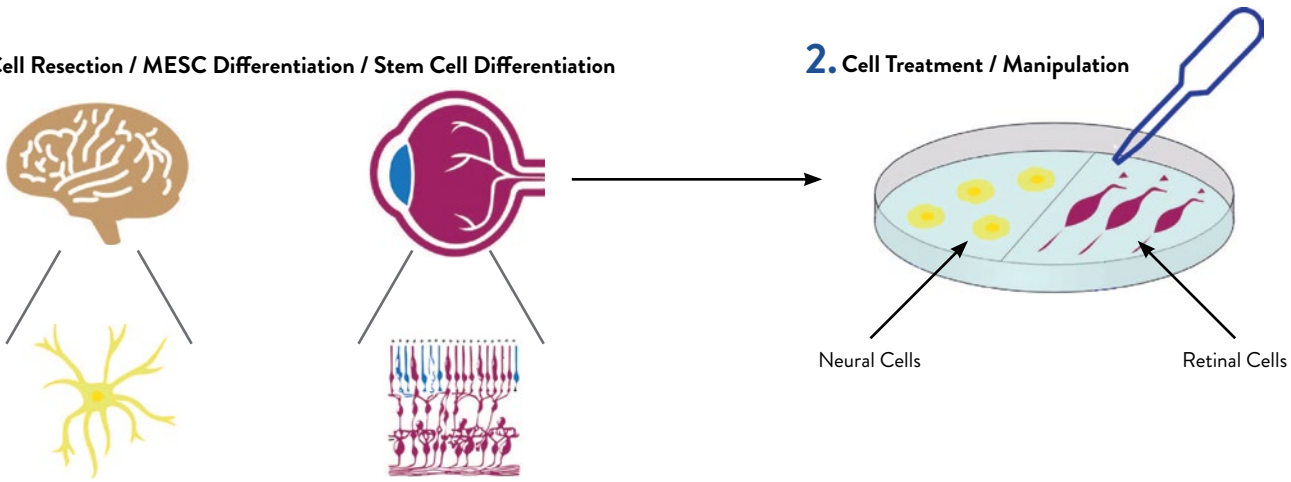
CLONAL ORGANOIDS FROM Lgr5+ CELLS



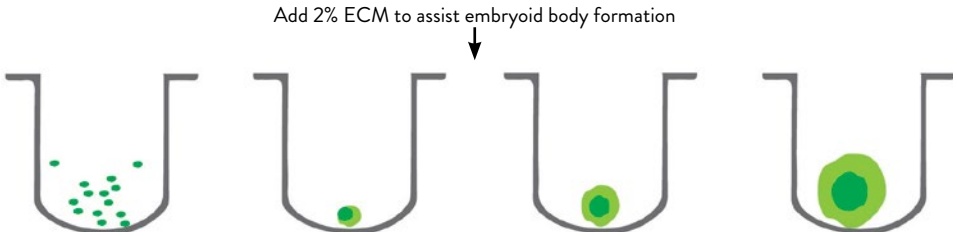
BRAIN AND RETINA ORGANOID FORMATION

1. **Cell Resection / MESC Differentiation / Stem Cell Differentiation**

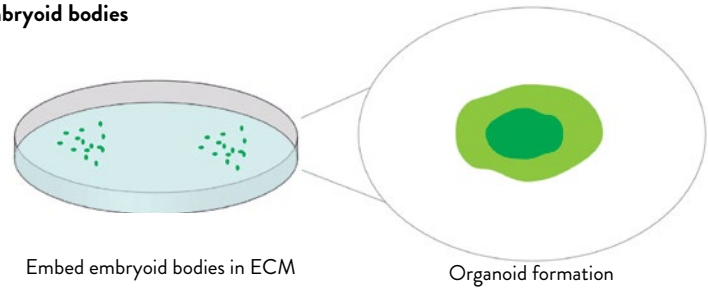
2. **Cell Treatment / Manipulation**



3. **Embryoid body formation in low-adhesion environment**



4. **Organoid formation from embryoid bodies**



Featured Products for Organoid Culture

Signaling molecules and extracellular matrices (ECMs) are used to influence cell growth, proliferation, differentiation and finally cell fate leading to the formation of a 3D self-organized tissue or organ-like structures (ie. organoids) with typical architecture characteristic for living tissues and organs.

AMSBIO offers a wide range of products needed to support organoid growth, including a variety of factors regulating cell transcription and ECMs. We also provide other reagents needed in organoid protocols, such as antibodies for tissue characterization, media and matrices for stem cell maintenance, cryopreservation solutions and organoid maintenance media (PBS).

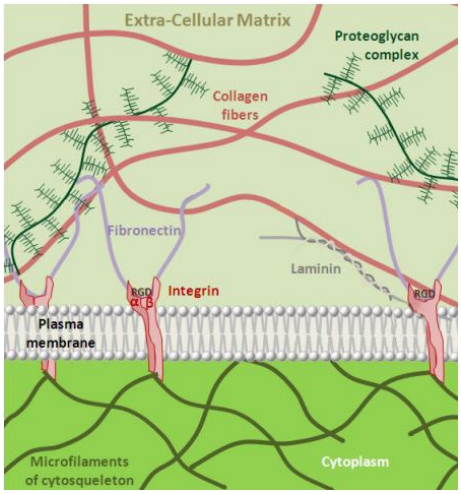


Figure 2: *Composition and structure of extracellular matrix.*

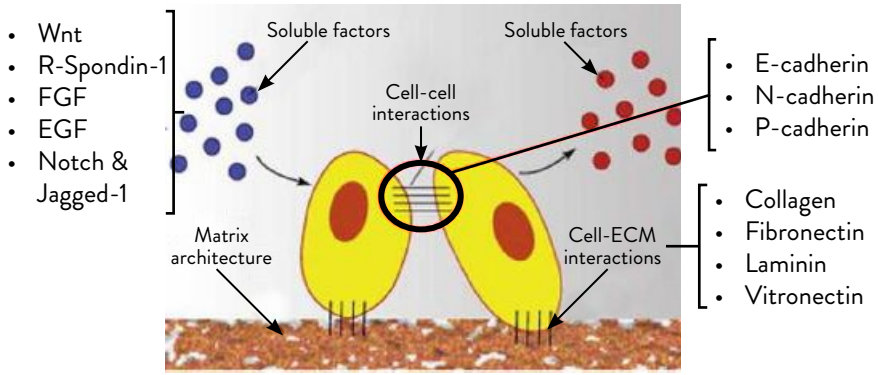


Figure 3: *Cells interactions and soluble factors.*

REGULATING CELL TRANSCRIPTION

We offer various factors for regulating cell transcription which are applicable in organoid cultures. They are involved in crucial signaling pathways controlling cell growth, proliferation and differentiation. Thus, manipulating your cell culture using these factors gives you the possibility to determine cell fate at a very high level.

When growing organoids from stem cells, different growth factor combinations are used to stimulate the signaling that takes place in the early phases of gestation. This enables the temporal control of cellular differentiation. Thus, the organoids develop from embryonic bodies in a similar way organs develop from embryonic tissue. The organoids obtained in this way have similar composition, architecture and tissue functions as the organ they recapitulate.

When growing organoids from tissue, it is important for the cells to grow, proliferate and migrate in the matrix. Controlling cell transcription is thus a huge issue when growing these types of organoids to ensure their development and health.

In this section, we highlighted some of our best products suitable for controlling cell transcription in organoid cultures. We offer a wide range of recombinant proteins and cell lines, including the CellExp™ line of recombinant proteins. These have higher biological activity and are all obtained from HEK293 cells. To view our full product range, please refer to our website or contact us with your specific requirements.

Wnt

Wnt proteins are a family of cysteine-rich secreted polypeptides (more than 16 mammalian family members) involved in several important cell functions such as proliferation, migration, polarity, cell-cell communication, survival and self-renewal. Wnt3a particularly plays an important role in the ability of organoids to expand. Additionally, loss of activation of Wnt expression is associated with alteration of cell morphogenesis, mutagenesis and finally cell fate. The Wnt canonical pathway can be seen in Figure 4. This clearly shows how Wnt activity leads to protein expression.

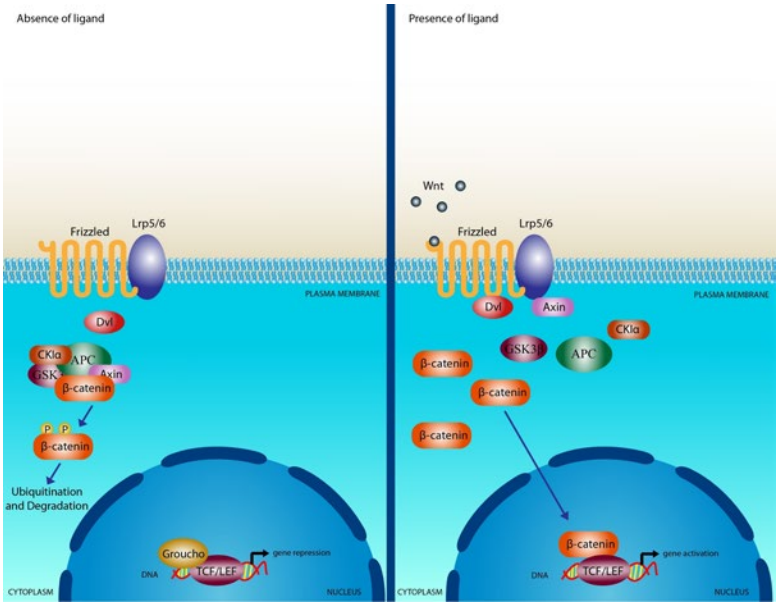


Figure 4. *Canonical Wnt signaling pathway. Wnt binds and activates the frizzled receptor (Lrp5/6 must be present for frizzled activation by Wnt). Lrp5/6 and frizzled interact with dishevelled (dvl) and axin, causing the dissociation of a complex phosphorylating beta-catenin, targeting it for degradation.*

SELECTED Wnt RECOMBINANT PROTEINS

AMSBIO offers a range of human and mouse Wnt recombinant proteins in low (75%) and high (85 – 90%) purity. These human recombinant proteins are purified from HEK293 cells while the mouse proteins are expressed in CHO cells. Both are suitable for various cell based assays and treatments.

Description	Purity	Pack Size	Catalogue No.
Mouse Recombinant Wnt3a	75%	2 µg	AMS.rmW3aL-002
		10 µg	AMS.rmW3aL-010
	85-90%	2 µg	AMS.rmW3aH-002
		10 µg	AMS.rmW3aH-010
Mouse Recombinant Wnt3a, with Wnt stabilizer	75%	2 µg	AMS.rmW3aL-002-stab
		10 µg	AMS.rmW3aL-010-stab
	85-90%	2 µg	AMS.rmW3aH-002-stab
		10 µg	AMS.rmW3aH-010-stab
Human Recombinant Wnt3a	75%	2 µg	AMS.rhW3aL-002
		10 µg	AMS.rhW3aL-010
	85-90%	2 µg	AMS.rhW3aH-002
		10 µg	AMS.rhW3aH-010
Human Recombinant Wnt3a, with Wnt stabilizer	75%	2 µg	AMS.rhW3aL-002-stab
		10 µg	AMS.rhW3aL-010-stab
	85-90%	2 µg	AMS.rhW3aH-002-stab
		10 µg	AMS.rhW3aH-010-stab

HIGHLY STABLE Wnt3a

Wnt3a protein is very unstable in serum-free medium, its half-life is about 2 hours. Our stabilizer significantly reduces Wnt3a protein aggregation, allowing to maintain its activity for 30 hours in serum free culture conditions (Figure 5). With the presence of Wnt protein stabilizer, purified Wnt3a protein can support even colon organoid cultures (requiring strong Wnt activity).

David Keller from Nexus Personalized Health Technologies in ETH Zurich purchased Wnt3a proteins from us. Here is what he says about our Wnt3a products:

"Your Wnt3a in the TOP/FOP Flash Reporter Assay Showed higher activity at the same concentration than the industry leader"

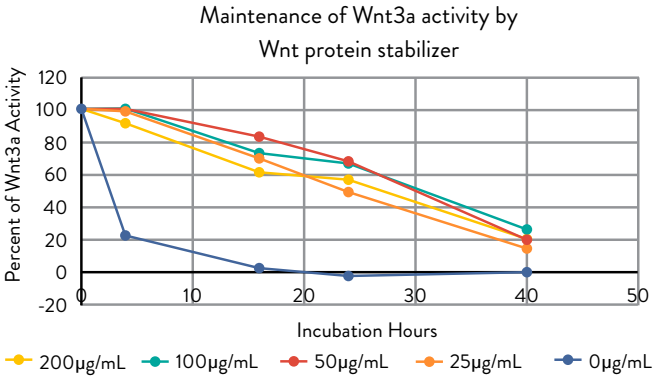


Figure 5. *Wnt3a half-life in serum free medium increases from around 2 hours in absence of a stabilizer (blue line) to 30 hours with increasing concentrations of stabilizer. Activity without incubation is set as 100% and background reading is set at 0%. The readings of Wnt3a activity from incubated (37 °C) samples are calculated as percentage of Wnt3a without incubation. Wnt3a activity was measured using TOP-Flash Reporter Assay. Measurements performed on NIH3T3 Wnt NIH3T3 Wnt reporter stable cell line.*

SELECTED Wnt REPORTER STABLE CELL LINES

Our Wnt reporter cell lines are available in multiple cell line, as different cell types have slightly different machineries for gene expression and regulation. For instance, HEK293 are "normal" cells, while HCT116 and SW480 are colorectal cancer cell lines.

HCT116 cells harbour a beta-catenin mutation. In normal cells, beta-catenin should be degraded quickly. However, this mutation results in accumulation of it. Accumulated beta-catenin move into nucleus and activates Wnt gene expression. SW480 harbours a APC mutation.

APC is a key protein in beta-catenin degradation complex. APC mutation results in accumulation of beta-catenin. SW480 cells have very strong Wnt signaling. Researcher can use these various cell lines to investigate the effects or targets of their Wnt signaling modulators.

In mammals, physiological Wnt signaling is intimately involved with the biology of adult stem cells and self-renewing tissues. The activity of canonical Wnt ligands is critical to successful organoid culture, especially to the one using Wnt3a-conditioned medium. We provide a very useful tool-Wnt gene reporter cells to evaluate Wnt3a activity. The Wnt reporter is HEK293 cell and T-cell effector-based and super sensitive to Wnt3a treatment. 10 ng/mL of Wnt3a treatment can bring more than 100-fold change of the canonical Wnt signal. The Wnt reporter cells have internal control-constant GFP expression, also have external controls-cells with mutation of T-cell effector binding element.

APPLICATIONS

- ✓ Evaluate Wnt protein bioactivity
- ✓ Screen anti-Wnt compounds/antibodies
- ✓ Screen Wnt signaling enhancer
- ✓ Evaluate Wnt protein stabilizer

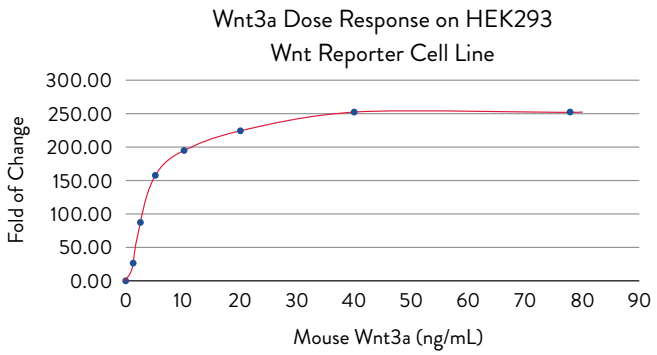


Figure 6. *Graph showing Wnt3a dose response on HEK293 Wnt Reporter cell line. Meaning of Fold of Change: the lumi reading without Wnt3a is set as a one (background reading). All other readings are divided by background reading.*

Description	Feature		Catalogue No.
Epithelial Wnt Reporter Cell Lines			
HEK293A Wnt Reporter Cell Line	Active	High Wnt Response	AMS.WRHEK293A-HWR
		High Endogenous Wnt Signal	AMS.WRHEK293A-HEW
	Mutant		AMS.WRHEK293M
Colorectal Cancer Wnt Reporter Cell Lines			
HCT116 Wnt Reporter Cell Line	Active		AMS.WRHCT116A
	Mutant		AMS.WRHCT116M
SW480 Wnt Reporter Cell Line	Active		AMS.WRSW480A
	Mutant		AMS.WRSW480M
Fibroblast Wnt Reporter Cell Lines			
NIH3T3 Wnt Reporter Cell Line	Active		AMS.WRNIH3T3A
Pre-Osteoblast Wnt Reporter Cell Lines			
MC3T3 E1 Wnt Reporter Cell Line	Active		AMS.WRMC3T3A

TITRATION OF Wnt CONDITIONED MEDIUM WITH THE TOP-FLASH ASSAY.

The TOP-Flash Assay, which is a Luciferase Reporter Assay, was used to monitor the concentration of Wnt3a recombinant protein in conditioned media, see Figure 7 for results.

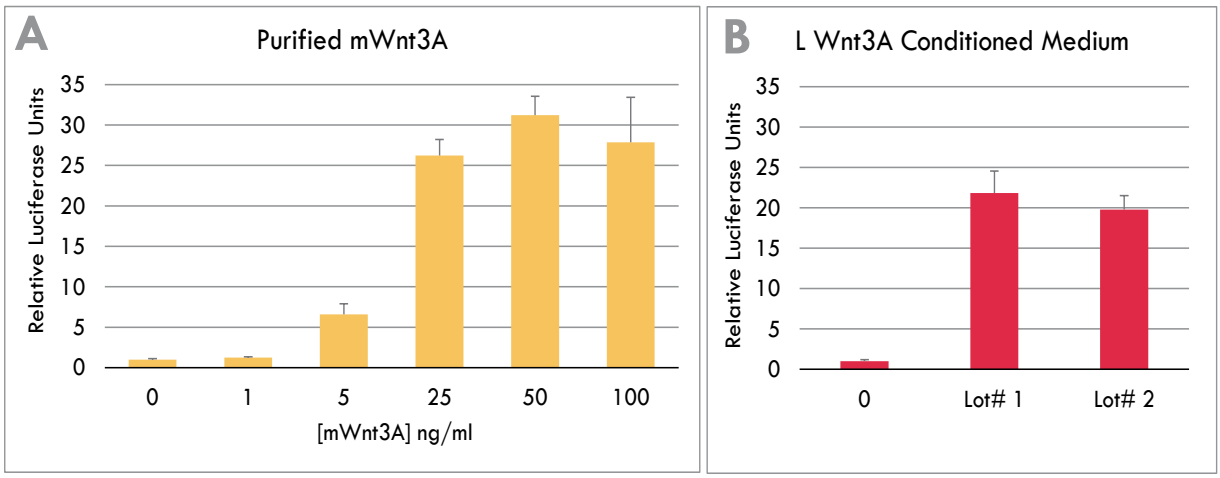


Figure 7: *A) Transfected HEK293 cells were exposed to different concentrations of recombinant murine Wnt3a protein, from 100 to 0 ng/ml diluted in Advanced DMEM/F12 with Glutamine. B) Two different preparations of L Wnt3a Conditioned Medium (produced with ATCC® CRL- 2647™) were diluted 1 to 2 in Advanced DMEM/F12 with Glutamine. The dose curve presented in panel A serves as a reference to compare the activity of Wnt3A conditioned media.*

-Personal communication - Gabe Benton

R-Spondin-1

Roof plate-specific Spondin-1 (R-Spondin-1 or RSPO1), also known as CRISTIN3, is a 27 kDa secreted activator protein that belongs to the R-Spondin family. R-Spondins positively regulate Wnt/β-catenin signaling, most likely by acting as a ligand for Lgr4-6 receptors and an inhibitor for ZNRF3. R-Spondin-1 induces proliferation of intestinal crypt epithelial cells, increases intestinal epithelial healing, and supports intestinal epithelial stem cell renewal. R-Spondin 1 is a critical ingredient used in the maintenance and proliferation of mouse and human organoid progenitor stem cells.

* Cited with Organoid Culture [5,6]

R-SPONDIN COMPARATIVE RESULTS

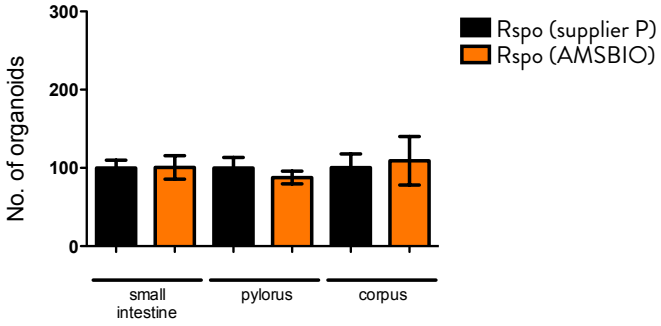


Figure 8. *Organoid counts of small intestine and gastric (pyloric and corpus) organoids at 4 days in culture using RSPO1 from AMSBIO and competitor.*

*-Data courtesy of Dr Nick Barker, A*STAR Insttute of Medical Biology, Singapore*

SELECTED R-Spondin-1 RECOMBINANT PROTEINS

Description	Pack Size	Catalogue No.
Human Recombinant R-Spondin-1	10 µg	7189-10
	50 µg	7189-50
	5 µg	AMS.PBG10386-U005
	20 µg	AMS.PBG10386-U020
	1 mg	AMS.RS1-H4221-1mg
	50 µg	AMS.RS1-H4221-50ug
Human CellExp™ R-Spondin-1	10 µg	7482-10
	10 µg	AMS.PBV11103R-10
Mouse Recombinant R-Spondin-1	50 µg	AMS.RS1-M5220-50ug
	1 mg	AMS.RS1-M5220-1mg
Purified Recombinant Protein of Human R-Spondin Homolog (Xenopus Laevis)	20 µg	TP723385

R-Spondin-1 (RSPO1) EXPRESSING CELL LINE

The 293T cell line is stably transfected to express murine RSPO1 with an N-terminal HA epitope tag and fused to a C-terminal murine IgG2a Fc fragment. This cell line is used to produce either purified RSPO1 or RSPO1 conditioned media. The murine RSPO1 protein has been used extensively in organoid culture to maintain Lgr5+ stem cells, and the FC and HA tags make it easy to purify or characterize.

BENEFITS

- ✓ Cell line expresses recombinant mouse RSPO1 protein
- ✓ Positively regulates Wnt/β-catenin signaling
- ✓ Essential medium component for most organoid culture models
- ✓ Purified protein and conditioned medium from our cell line has been used for culturing both human and mouse organoids.

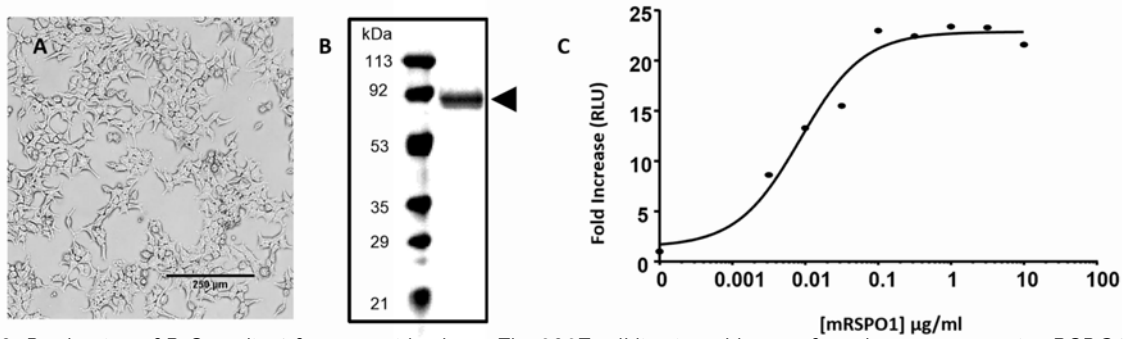


Figure 9. *Production of R-Spondin-1 for organoid culture. The 293T cell line is stably transfected to express murine RSPO1 with an N-terminal HA epitope tag and fused to a C-terminal murine IgG2a Fc fragment. A) The HA-R-Spondin-1- Fc 293T cell line is cultured with zeocin to select for stably transfected cells. B) Production of HA-R-Spondin-1-Fc is characterized using Western Blot for R-Spondin1 protein (arrow). C) HA-R-Spondin-1-Fc induces activaton of Wnt/ß-catenin response when evaluated using the Top-Flash Luciferase Assay.*

Description	Pack Size	Catalogue No.
R-Spondin (RSPO1) Expressing Cell Line	1 vial	3710-001-K

FGF

Fibroblast growth factors (FGFs) are a family of functional proteins including 22 members. FGFs act at 4 different types of receptors initiating different tissue processes. FGFs are important in early development contributing to mesoderm induction, limb development, neural induction and neural development. In mature tissues, FGFs are known to be crucial for angiogenesis, keratinocyte organization and wound healing processes.
The FGF pathways have been successfully manipulated to obtain organoids from tissues and stem cells. Below is a list of some of our human FGFs:

SELECTED FGF RECOMBINANT PROTEINS

Peptide	Description	Pack Size	Catalogue No.
FGF-2 (basic FGF)	Human	50 µg	4037-50
		1 mg	4037-1000
	Animal-Free	10 µg	AMS.480-10
		100 µg	AMS.480-100
FGF-4	Human	25 µg	4043-25
		1 mg	4043-1000
	Animal-Free	25 µg	AMS.PBG10501-U025
		100 µg	AMS.PBG10501-U100
FGF-9	Human	20 µg	4056-20
		1 mg	4056-1000
	Animal-Free	20 µg	AMS.PBG10498-U020
		100 µg	AMS.PBG10498-U100
FGF-10	Human	5 µg	AMS.PBG10091-U005
		25 µg	AMS.PBG10091-U025
	Animal-Free	25 µg	AMS.PBG10500-U025
		100µg	AMS.PBG10500-U100
FGF-19	Human	25 µg	4542-25
		1 mg	4542-1000

FGF RECOMBINANT PROTEINS OF OTHER SPECIES ARE AVAILABLE AS WELL

EGF

Epidermal Growth factor (EGF) is a potent growth factor, which stimulates the proliferation of a wide variety of epidermal and epithelial cells. Additionally, EGF has been shown to inhibit gastric secretion, and to be involved in wound healing. EGF signals through a receptor known as cerbB which is a class I tyrosine kinase receptor. This receptor also binds with TGFα and VGF (vaccinia virus growth factor). Recombinant Human EGF is a 6.2 kDa globular protein containing 53 amino acid residues including 3 intramolecular disulphide bonds.

Description	Pack Size	Catalogue No.
Human Recombinant Epidermal Growth Factor (EGF)	100 µg	4022-100
	1 mg	4020-1000
Animal-Free Recombinant Human EGF	100 µg	AMS.PBG10490-U100
	500 µg	AMS.PBG10490-U500
Human CellExp™ EGF	10 µg	AMS.PBV10858R-10
Mouse Recombinant EGF	100 µg	TP723069

Notch and Jagged-1

The Notch signaling pathway controls cell fate in vertebrate and invertebrate tissues. Notch signaling is triggered through the binding of a transmembrane ligand to Notch transmembrane receptor on a neighbouring cell. This results in proteolytic cleavage of the Notch receptor, releasing the constitutively active intracellular domain of Notch (NICD). Translocating to the nucleus, NICD associates with transcription factors to turn on transcription of Notch-responsive genes.

Jagged-1 is one of the cell surface proteins that interacts with the Notch receptor. The Notch signaling pathway is highly conserved evolutionarily, controlling cell fate through interactions with transcription factors.

Description	Pack Size	Catalogue No.
Notch-1, Mouse Recombinant	25 µg	7590-25
Notch-2, Mouse Recombinant	25 µg	7531-25
Human Jagged 1 (JAG1) Protein	50 µg	AMS.JA1-H52H9-50ug
	1 mg	AMS.JA1-H52H9-1mg
Notch Signaling Pathway Notch1/CSL Reporter - HEK293 Cell Line	2 vials	60652

BMP and Noggin

Bone Morphogenetic Proteins (BMPs) are a group of growth factors that play a crucial role in development. BMPs are also part of the TGF-β cytokine superfamily, initiating SMAD and NF-κB mediated transcription. BMPs play a crucial role in neuronal cell differentiation from progenitor cells. Thus, BMP activation is desired for the generation of brain and neuronal-associated organoids. Selective BMP inhibition and stimulation can drive cells toward tissue-specific progenitors (eg. stomach, liver, kidney). Noggin is an endogenous BMP receptor antagonist.

Noggin belongs to a group of diffusible proteins which bind to ligands of the TGF-β family and regulate their activity by inhibiting their access to signaling receptors. Noggin was originally identified as a BMP-4 antagonist whose action is critical for proper formation of the head and other dorsal structures. Consequently, Noggin has been shown to modulate the activities of other BMPs including BMP-2,-7,-13, and -14.

Recombinant human Noggin protein is a 23.1 kDa non-disulphide-linked homodimer consisting of a total of 206 amino acid residues.

SELECTED BMP-4 AND Noggin RECOMBINANT PROTEINS

Description	Pack Size	Catalogue No.
BMP-4 Human Recombinant	10 µg	4578-10
	1 mg	4578-1000
Human CellExp™ BMP-4	10 µg	AMS.PBV10677r-10
	50 µg	AMS.PBV10677r-50
Animal-Free Recombinant Human BMP-4	10 µg	AMS.PBG10504-U010
	100 µg	AMS.PBG10504-U100
BMP-4 Blocking Peptide	50 µg	5674RBP-50
Human Recombinant Noggin	100 µg	4675-100
	1 mg	4675-1000
Human CellExp™ Noggin	10 µg	6474-10
	50 µg	6474-50
Purified Recombinant Protein of Human Noggin (NOG)	20 µg	TP723333
Purified Recombinant Protein of Mouse Noggin (NOG)	20 µg	TP723334

Lgr5 & Other Antibodies

Leucine-rich containing G-protein coupled receptor 5 (Lgr5) is an orphan G-protein coupled receptor. The structure of Lgr5 is highly conserved across different species. While Lgr5 is an orphan receptor because a specific ligand has not yet been identified, it is known that it is part of the Wnt signaling cascade. R-Spondin-1 and Wnt3a can bind Lgr5 to trigger its internalization.

Lgr5 is a marker of adult stem cells. It is considered that a 3D culture has become organoid when it starts expressing Lgr5. Thus, staining for Lgr5 is common when working with organoids. We offer Lgr5 antibodies from a wide range of clones, including the OTI2A2 clone. The OTI2A2 clone is the most trusted and widely cited Lgr5 antibody. It has high reproducibility, broad applications and it comes in convenient formats (carrier free & different conjugations available).

Lgr5 ANTIBODIES

Clone	Pack Size	Catalogue No.
OTI202	100 µl	TA503316
UMAB212	30 µl	UM870104
	100 µl	UM800104
UMAB210	30 µl	UM970102
	100 µl	UM800102
UMAB211	30 µl	UM870103
	100 µl	UM800103
OTI7F2	100 µl	TA808752
OTI3F1	100 µl	TA808748

OTHER ANTIBODIES

Gene	Pack Size	Catalogue No.
OCT4	100 µg	AMS.AO1317a
SOX2	100 µg	AMS.AO1218a
TBX6	100 µg	AMS.AT4172a
HOXD11	100 µg	AMS.AT2426a
PAX8	100 µg	AMS.BT-MCA0576

COLLAGEN - EXTRACELLULAR MATRIX PROTEIN

Collagen is the main structural protein in the extracellular space in the various connective tissues in animals. As the most abundant protein in mammals, it makes up 25% to 35% of the whole body proteins content. Collagen, in the form of elongated fibrils, is mostly found in fibrous tissues such as tendons, ligaments and skin. It is also abundant in corneas, cartilage, bones, blood vessels, the gut, intervertebral discs and the dentin in teeth. Collagen constitutes one to two percent of muscle tissue, and accounts for 6% of the weight of strong, tendinous muscles. Fibroblasts are the most common cells that create collagen.

Collagen I

Type I collagen is a major structural component of skin, bone, tendon, and other fibrous connective tissues, and differs from other collagens by its low lysine hydroxylation and low carbohydrate composition. Type I collagen is a hetero-trimer composed of two α1 chains and one α2 chain, which spontaneously form a triple helix scaffold at neutral pH and 37 °C. This phenomenon can be exploited to promote cell attachment, proliferation, differentiation, migration, and tissue morphogenesis during development. The three dimensional (3D) collagen gels simulate the *in vivo* cell environment better than traditional 2D systems. This allows Type I collagens to be very useful in studying cell function and behavior, and the effects of diseases on the mechanical properties of the ECM and the interactive cells.

Collagen III

Type III collagen provides structure and strength to connective tissue. It is found in many places in the body, especially skin, lung, intestinal walls and the walls of blood vessels. Collagen III is initially produced as procollagen, which is then modified by the cell using specific enzymes to enable the formation of a stable molecule in order to crosslink it to other molecules outside the cell. Type III Collagen is typically used as a thin coating on tissue culture surfaces and acts as a substrate scaffold to enhance cell attachment, adherence and proliferation.

SELECTED RECOMBINANT Collagen I

Peptide	Description	Pack Size	Catalogue No.
Cell Culture Grade Collagen I	Bovine	4 mg/ml x 12.5 ml	1202
	Porcine	4 mg/ml x 12.5 ml	1203
Recombinant Collagen I	Human	2 mg	4796-2
		10 mg	AMS.PBV10415r-10
Attachin™ Collagen I	Bovine	3 mg/ml x 100 ml	AMS.Q1BC1000
		5 mg/ml x 35 ml	AMS.Q1BC1G35
		6 mg/ml x 50 ml	AMS.Q1BC0500
		10 mg/ml x 20 ml	AMS.Q1BC0200

SELECTED RECOMBINANT Collagen III

Peptide	Pack Size	Catalogue No.
Human Recombinant Collagen III	1 mg	AMS.PBV10416r-1
	5 mg	AMS.PBV10416r-5
Attachin Human Collagen III (75% Collagen III, 25% Collagen I)	1 mg/ml x 10 ml	AMS.Q3HC0100

All Attachin™ Collagen products are isolated from specific tissues and are purified using a validated manufacturing process that insures inactivation of possible prion and/or viral contaminants. Attachin™ Collagens are then sterilized by membrane filtration and confirmed negative for bacterial and fungal contaminants. Identities and purities of collagens are determined by SDS-PAGE gel electrophoresis.

Collagen I Coated Products

Description	Pack Size	Catalogue No.
6-well rat-tail Collagen I coated plate	5 pack	CC-6
12-well rat-tail Collagen I coated plate	5 pack	CC-12
24-well rat-tail Collagen I coated plate	5 pack	CC-24
96-well rat-tail Collagen I coated plate	5 pack	CC-96
T-25 rat-tail Collagen I coated flask	5 pack	CC-25
T-75 rat-tail Collagen I coated flask	5 pack	CC-75
T-225 rat-tail Collagen I coated flask	1 pack	CC-225

REAGENTS AND LABWARE

Lipidure®- Ultra Low Adhesion Products

Spheroid cell culture is typically based on the spontaneous formation of an aggregate of cells in an environment where cell-cell interactions dominate over cell-substrate interaction. This can be achieved by using low-attachment cell culture conditions. Lipidure®-COAT plates and dishes are a top of the range solution for spheroid formation, with the Lipidure® coating providing a superior low-attachment solution for the formation of single spheroids in each well of multi-well plates. Lipidure®-COAT plates have been successfully used to make embryoid bodies that were then differentiated into organoids.
*Cited with Organoid Culture [7-11]

Description	Pack Size	Catalogue No.
Lipidure®-Coat Low Adhesion Plate A-U96 (96 well U-bottom plate)	6 plates	AMS.LCP-A-U96-6
Lipidure®-Coat Low Adhesion Plate A-V96 (96 well V-bottom plate)	6 plates	AMS.LCP-A-V96-6

See application data on page 35 and 35

iMatrix Recombinant Laminin E8 Fragments

iMatrix-511 is an innovative cell culture matrix compatible with a wide variety of cell types, and exceptionally well suited for PSCs. This product is composed from recombinant Laminin-511 E8 proteins fragments which:

- ✓ allow ES/iPS cells to be maintained in xeno-free culture conditions,
- ✓ enable passage of single cells,
- ✓ provide greater adhesion than full-length Laminin, Vitronectin, BME or Matrigel.

Using iMatrix-511 for ES/iPS cell culture is highly efficient, as it can be used with a pre-mix method where plates do not need to be coated, saving time, materials and money. iMatrix-511 shows better adhesion activity when the pre-mix method is used.
* Cited with Organoid Culture [12-15]

StemFit®

For the best results, we strongly recommend iMatrix-511 be used together with StemFit® medium

Description	Pack Size	Catalogue No.
iMatrix-511	350 µg (175 µg x 2 tubes)	AMS.892 011
iMatrix-511	1050 µg (175 µg x 6 tubes)	AMS.892 012
iMatrix-511 silk	1050 µg (175 µg x 6 tubes)	AMS.892 021
iMatrix-411	350 µg (175 µg x 2 tubes)	AMS.892 042
iMatrix-411	1050 µg (175 µg x 6 tubes)	AMS.892 041

StemFit® - Feeder Free Stem Cell Culture Medium

StemFit® is a highly stable xeno free, defined medium proven to effectively maintain iPS and ES cells under feeder-free conditions during the reprogramming, expansion and differentiation phases of stem cell culture. StemFit® combines high colony forming efficiency with lower than standard medium volume consumption to offer cost effective colony expansion when compared to leading competitors. This makes StemFit® an ideal stem cell culture medium.

Morizane and Bonventre (2017) [15] used StemFit® to passage human pluripotent stem cells (hPSCs) in a protocol to obtain kidney progenitor cells and kidney organoids from hPSCs.
* Cited with Organoid Culture [12-15]

iMatrix-511

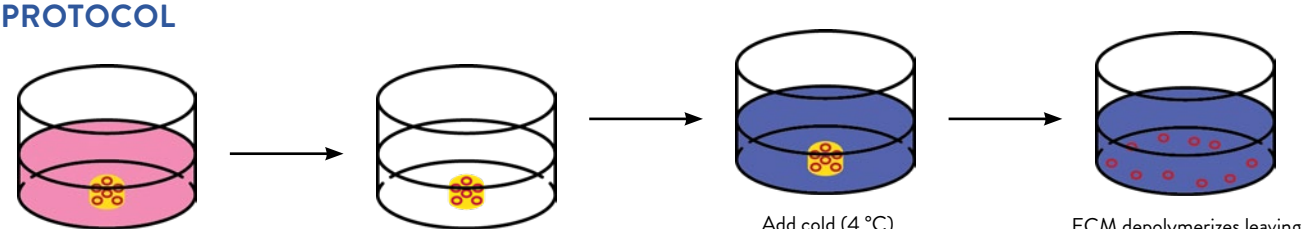
For the best results, we strongly recommend StemFit® medium be used together with iMatrix-511.

Description	Pack Size	Catalogue No.
StemFit®	500 ml	SFB-500

Organoid Harvesting Solution

Organoid culture exhibits cellular behavior and morphology similar to those seen *in vivo*. However, the adaptation of this model for studying biochemical processes has been impeded by the challenge of separating intact organoids from extracellular proteins comprising the hydrogel. Commonly, proteases are employed to degrade these extracellular proteins, however, proteases also degrade proteins on the cell surface and protease activity may carry over into subsequent cultures or lysate preparations. Organoid Harvesting Solution provides a ready to use, non-enzymatic method for depolymerizing extracellular matrix proteins to allow for harvesting of intact organoids for passaging, cryopreservation, or biochemical analysis.
* Cited in Organoid Culture [16]

PROTOCOL



BENEFITS

- ✓ Ready to use
- ✓ Non-enzymatic chelating solution
- ✓ Depolymerizes basement membrane matrix for harvesting organoids from culture
- ✓ Gentle for cells: preserves original morphology

APPLICATIONS

- ✓ Organoid passaging
- ✓ Sample preparation (PCR, Western Blot, and Immunohistochemistry)

Description	Pack Size	Catalogue No.
Organoid Harvesting Solution	100 ml	3700-100-01

See application data on page 32

RNA-STAT 60 - RNA, DNA and Protein Isolation Reagent

RNA-STAT 60 is a complete and ready to use reagent for isolation of total RNA, DNA and protein from cells and tissues of human, animal, plant, yeast, bacterial, and viral origin. Extensive laboratory tests have shown that the RNA-STAT 60 is highly reliable and producing consistent results.

The composition of RNA-STAT 60 includes phenol and guanidinium thiocyanate in a mono phase solution. A biological sample is homogenized in the RNA-STAT 60 using a glass-Teflon or Polytron homogenizer. Upon addition of chloroform, the homogenate separates into two phases: aqueos phase and organic phase. The total RNA remains exclusively in the aqueous phase while DNA and proteins are extracted into an organic phase and interphase. The total RNA is precipitated from the aqueous phase by addition of isopropanol, then washed with ethanol and solubilized in water. The entire procedure for total RNA isolation can be completed in 1 hour. Using RNA-STAT 60 is the most effective single step method of total RNA isolation. The recovery of **undegraded mRNAs** using RNA-STAT 60 is **30-150 % greater** than with any other method of RNA isolation.

BENEFITS

- ✓ Total RNA / mRNA in under 60 minutes
- ✓ Northern blot / PCR – ready mRNA in under 60 minutes
- ✓ No further purification required for use in subsequent procedures including Northern Blotting and PCR
- ✓ Extracts 30-150% more total RNA / mRNA than any other method
- ✓ Cost effective method requiring less reagent/sample

The total RNA isolated by the RNA-STAT 60 is undegraded and free of protein and DNA contamination. It can be used for Nothern analysis, dot blot hybridization, poly A+ selection, *in vitro* translation, RNase protection assay, molecular cloning , and for polymerase chain reaction (PCR) without additional treatment with DNase. The simplicity of the RNA-STAT 60 makes it possible to process simultaneously a large number of samples, and the excellent recovery of RNA from very small biological samples (biopsies, etc).
* Cited with Organoid Culture [17]

Description	Pack Size	Catalogue No.
RNA-STAT 60	100 ml	CS-110
RNA-STAT 60	200 ml	CS-111
RNA-STAT 60	500 ml	CS-502

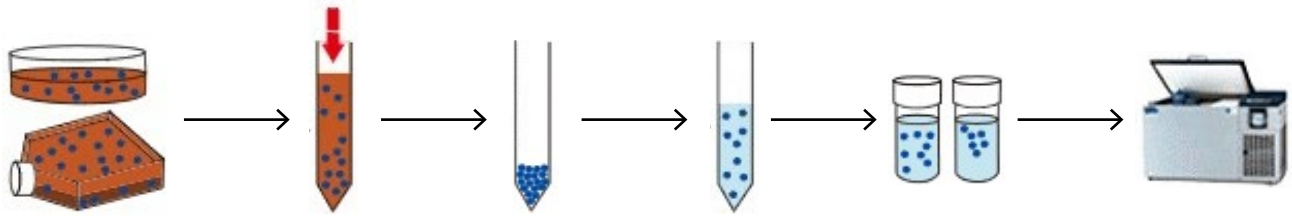
RNA-Bee - RNA Isolation Reagent

RNA-Bee is a complete and ready to use reagent for isolation of total RNA from cells and tissues from samples of human, animal, plant, bacterial, and viral origin. Isolation of RNA using RNA-Bee is the improved version of the single step method. The improved RNA-Bee provides a fast and highly reliable method for isolating pure and undegraded RNA from a large variety of biological samples. RNA-Bee is a mono phase solution containing phenol and quanidine thiocyanate. A biological sample is homogenized or lysed in RNA-Bee and the homogenate/lysate is separated into aqueous and organic phases by the addition of chloroform. The subsequent centrifugation efficiently removes DNA and proteins from the aqueous phase containing RNA. The undegraded, pure RNA is obtained from the aqueous phase by the isopropanol precipitation, then washing with ethanol and solubilisation in an appropriate solution. The entire isolation procedure can be completed in 1 hour. The isolated RNA is appropriate for Nothern blotting, poly A + selection, RT-PCR, and other molecular biology techniques.

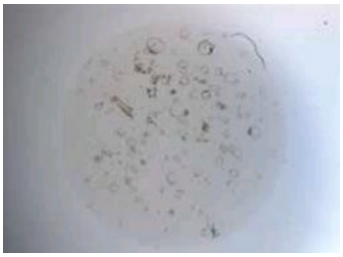
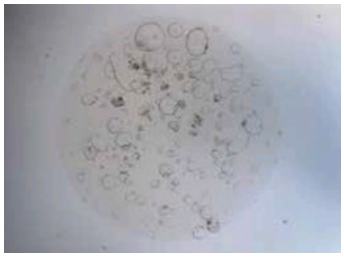
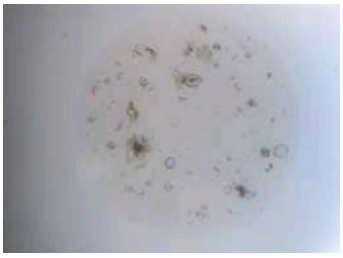
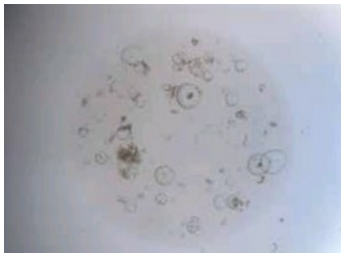
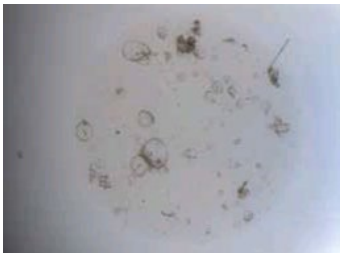
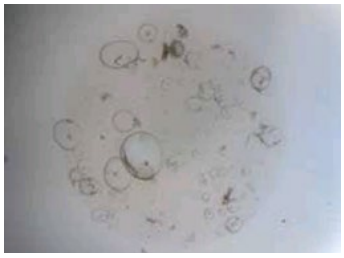
Description	Pack Size	Catalogue No.
RNA-Bee	100 ml	CS-104B
RNA-Bee	200 ml	CS-105B
RNA-Bee	500 ml	CS-501B

CELLBANKER® Freezing Media Series

CELLBANKER® is a series of easy to use cell cryopreservation media. CELLBANKER® enables long term storage of different type of cells maintaining consistent high cell viability regardless of their sensitivity due to superior protection against cell stress during freeze/thaw cycles . As cell freezing medium, CELLBANKER® does not require a gradual temperature decrease in programmed freezer nor storage in liquid nitrogen to guaranty efficient cell storage. This makes CELLBANKER® more affordable and accessible than other cell freezing media. CELLBANKER® solutions are simple to use and allow to achieve the highest cell viability while maintaining cells natural functions.



Human Liver Organoids Stored in CELLBANKER®

	Day 2	Day 4	Organoid Growth Following Freezing
CELLBANKER® 1			☑
CELLBANKER® 2			☑
STEM-CELLBANKER® GMP grade			☑

Images courtesy of Robert Arnes, Huch Lab

"Day 2 and day 4 images clearly show that organoids recovered and grew well in these three CELLBANKER® freezing media"

-Meritxell Huch, Gurdon Institute, Cambridge, UK

CELLBANKER® 1

The first product of the CELLBANKER® series, CELLBANKER® 1, was launched in 1992 and now has a significant history of reliable,consistent and high viability recoveries post-cryopreservation.
Contains serum, DMSO, glucose, salts and buffer.

Description	Pack Size	Catalogue No.
CELLBANKER® 1	20 ml	11889
CELLBANKER® 1	4 x 20 ml	11884
CELLBANKER® 1	100 ml	11888

CELLBANKER® 2

CELLBANKER® 2 is a serum free cell freezing medium, which formulation is optimised for serum free cultured cells and peptide/protein expressing cells. As it does not contain any animal derived products it is recommended for all applications where risks of contamination must be avoided.
Contains no animal derived products, fully defined.
*Cited with Organoid Culture [18]

Description	Pack Size	Catalogue No.
CELLBANKER® 2	20 ml	11892
CELLBANKER® 2	4 x 20 ml	11893
CELLBANKER® 2	100 ml	11891

STEM-CELLBANKER® (GMP GRADE) - DMF with FDA

STEM-CELLBANKER® is a chemically defined, xeno free freezing medium manufactured in compliance with JPN, EU, US, and PIC/S GMP guidelines - optimized for stem cells and iPS cells storage as well as other valuable cells.

Available in DMSO and DMSO free formulations STEM-CELLBANKER® is completely free of serum and animal derived components and contains only European or US Pharmacopoeia graded ingredients. STEM-CELLBANKER® is ready to use and requires no special devices, such as a controlled rate freezer, in order to achieve consistently high cell viability following resuscitation from cryopreservation, even over extended long term storage. STEM-CELLBANKER® significantly increases cell viability while maintaining cell pluripotency, normal karyotype and proliferation ability after freeze-thaw. It is an optimal freezing. Cryopreservation of cells using STEM-CELLBANKER® is an optimal solution for basic research and in the clinical application of cell therapy products.

Description	Pack Size	Catalogue No.
STEM-CELLBANKER® - GMP	20 ml	11897
STEM-CELLBANKER® - GMP	4 x 20 ml	11894
STEM-CELLBANKER® - GMP	100 ml	11890
STEM-CELLBANKER® - GMP - DMSO Free	20 ml	11897F
STEM-CELLBANKER® - GMP - DMSO Free	4 x 20 ml	11894F
STEM-CELLBANKER® - GMP - DMSO Free	100 ml	11890F

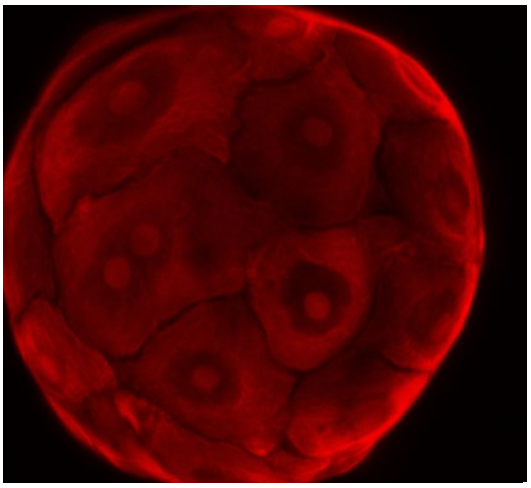
Organoid Culture Examples

LIVER ORGANOID CULTURE

Human Liver

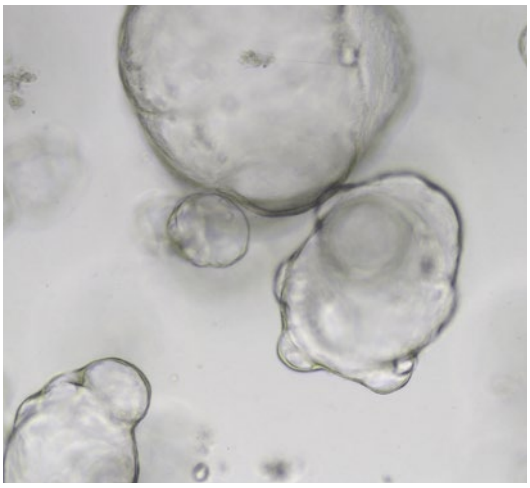
In their paper in Cell, Huch and Clevers et al (2015) demonstrated that:

- ✓ Primary human bile duct cells can readily be expanded into 3D liver organoids *in vitro* using ECM
- ✓ Adult liver stem cells maintain self-renewal capacity, differentiate into functional hepatocytes *in vitro* and generate bona fide hepatocytes upon *in vivo* transplantation.
- ✓ Expanded cells preserve their genetic integrity over months in culture (agreeing with the authors' previous observations in a mouse model).
- ✓ Organoids derived from patients with genetic disorders can be used to model liver disease *in vitro*.



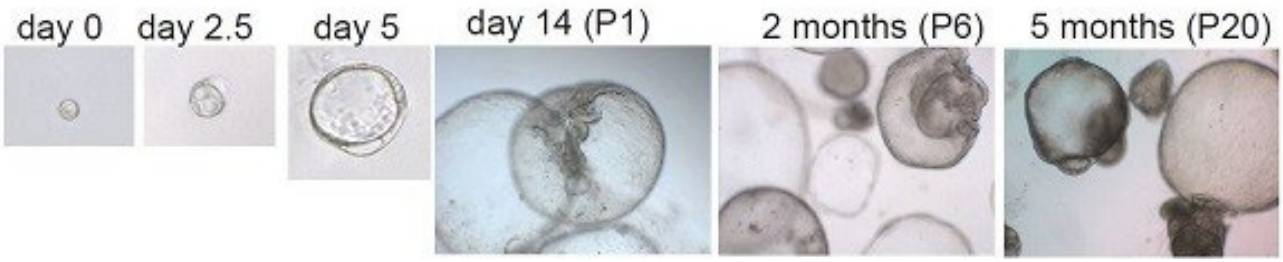
Marker Expression in Human Liver Organoids on ECM from AMSBIO.

Image courtesy of Helmuth Gehart/Professor Hans Clevers, Hubrecht Institute, Utrecht, Netherlands



Differential interference contrast image of human organoids grown in ECM and cultured for more than 2 months in human liver complete medium. Magnification, 4x.

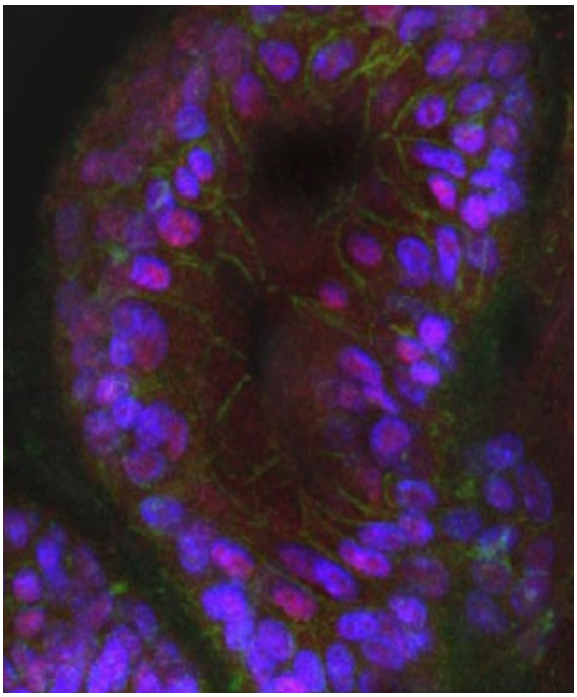
Image courtesy of Meritxell Huch, Gurdon Institute, University of Cambridge, UK



Long-term culture of Human Liver Organoids on ECM RGF.

(Clonal cultures obtained by seeding sorted cells at one cell per well)

Image courtesy of Meritxell Huch, Gurdon Institute, University of Cambridge, UK



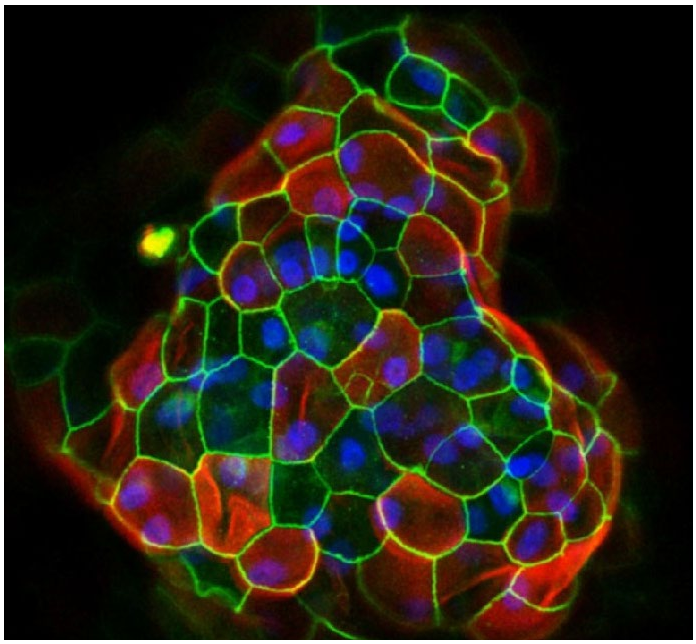
Marker Expression in Human Liver Organoids on ECM.

Confocal image stained for ECAD (green) and the hepatocyte marker HNF4 (red); nuclei counter-stained with Hoechst (Blue).

Image courtesy of Dr. Meritxell Huch, Gurdon Institute, University of Cambridge, UK

"We have obtained culture conditions that allow us to long-term expand genetically stable human donor liver cells in organoid culture. One of the clues to this success is the use of ECM that allows the cells to grow in 3D."

Dr. Meritxell Huch (Gurdon Institute, University of Cambridge, UK)

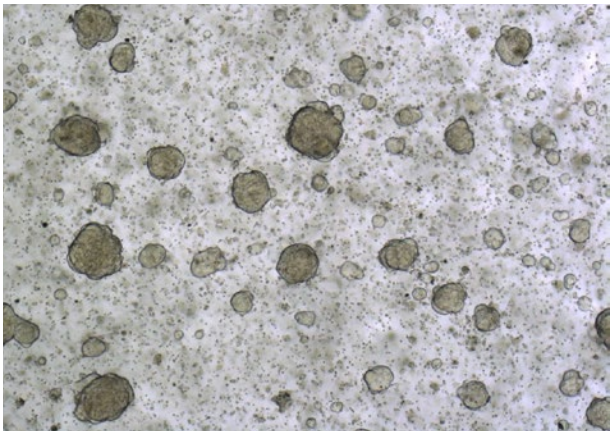


Differentiation of Organoids into Hepatocytes on ECM from AMSBIO.

Expression of hepatocyte genes in human liver organoid after 11 days on differentiation medium. Immunofluorescence for albumin (ALB, red) and ZO-1 (green); nuclei counterstained with Hoechst (blue)

Image courtesy of Dr. Meritxell Huch, Gurdon Institute, University of Cambridge, UK

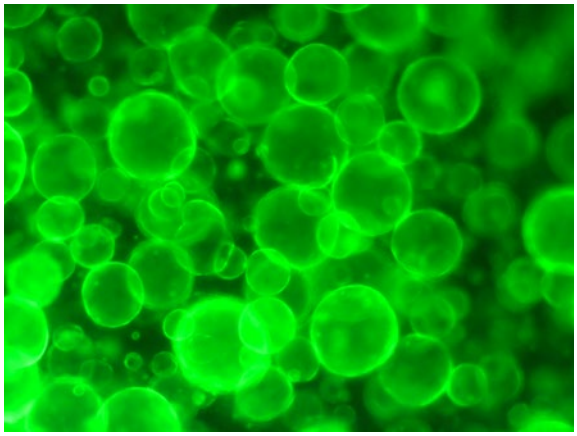
Hepatocellular Carcinoma (HCC)



Hepatocellular carcinoma (HCC) organoid model, grown in ECM.

Image courtesy of Prof. Dr. med. Markus Heim, Hepatology Group, Department of Biomedicine, University Hospital Basel, Basel, Switzerland.

Mouse Liver



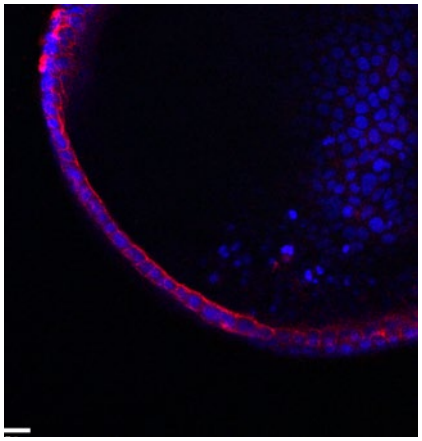
GFP-labeled organoids, grown from mouse liver stem cells and grown on ECM from AMSBIO.

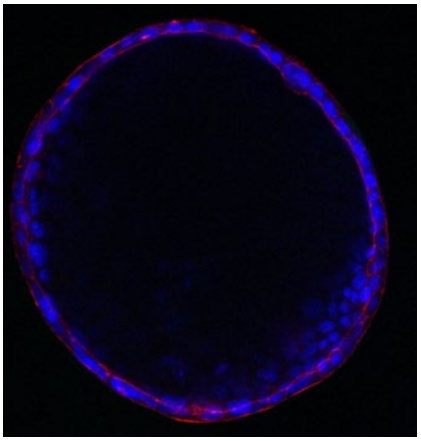
The cells were infected with a lentivirus expressing GFP

Image courtesy of Dr. Derk ten Berge, Erasmus MC Stem Cell Institute, Rotterdam, Netherlands

INTESTINAL ORGANOID CULTURE

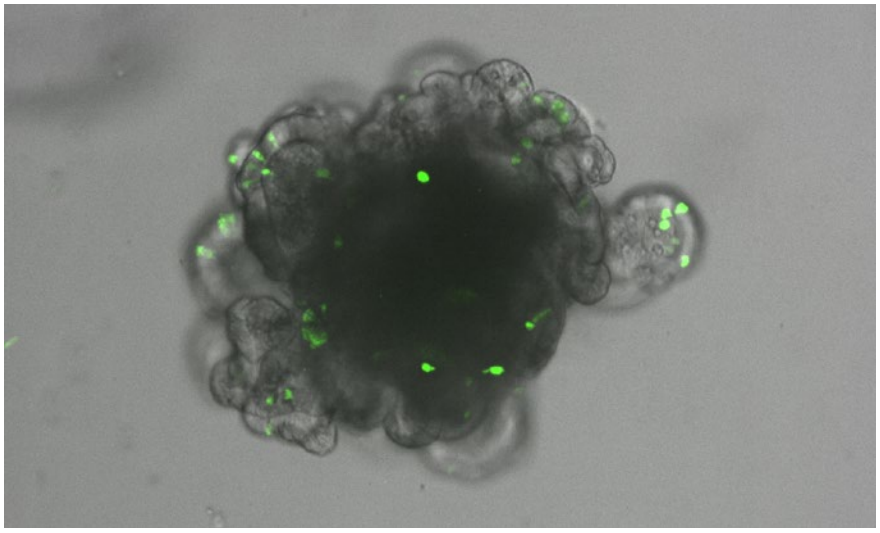
Small Intestine Organoids





Small intestine organoids with DNA in blue and structural proteins in red, grown on ECM.

Images courtesy of J Wosen, E Mellins Lab, Stanford University, USA.

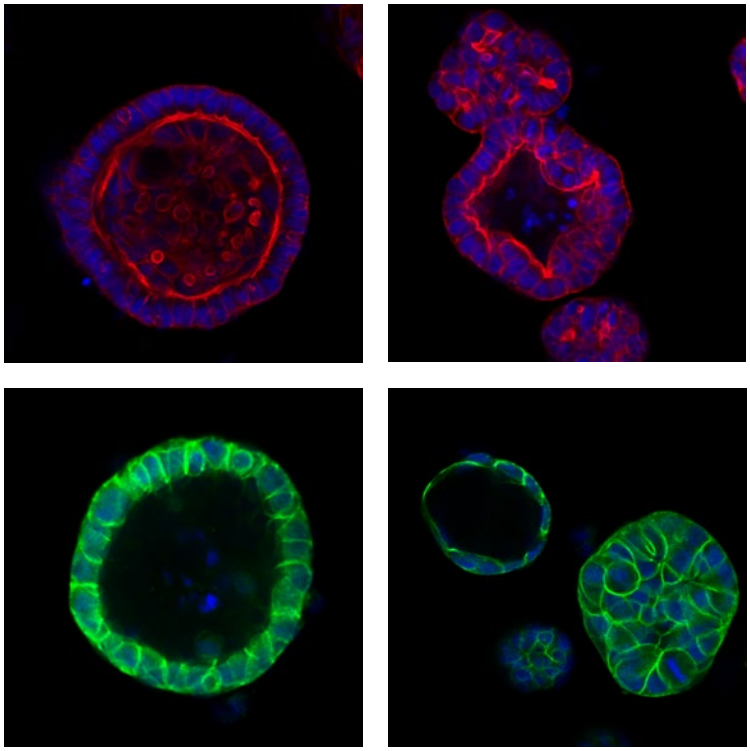


Small Intestine Organoid Culture.

Live fluorescence image of small intestinal organoids genetically modified to express EYFP when CCK (cholecystokinin) is expressed.

Image courtesy of Patricia Fonseca Pedro, Gavin Bewick lab, Diabetes & Nutritional Sciences, KCL.

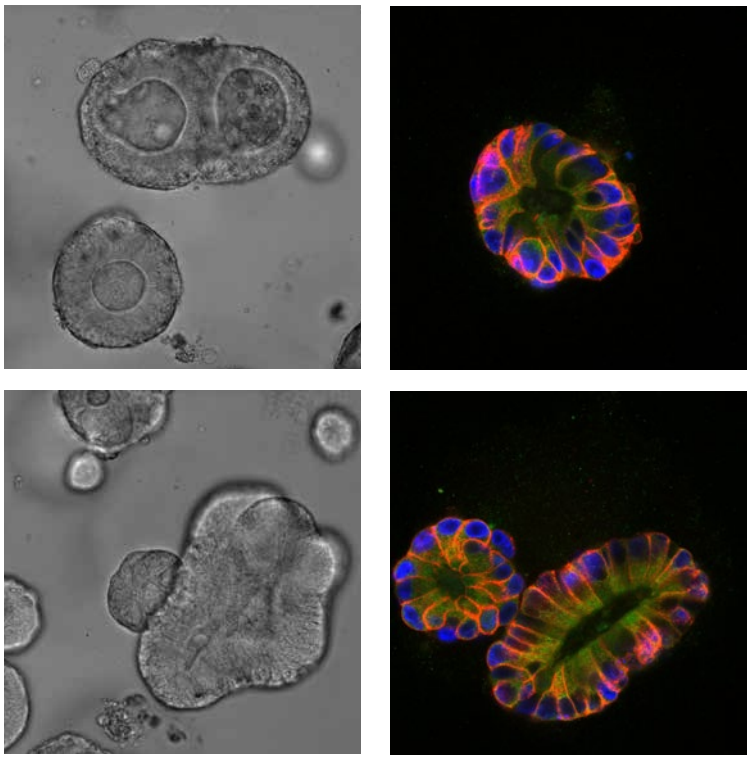
Human Colorectal



Human Colorectal Cancer (CRC) organoids grown from single cells on ECM.

Immunofluorescence for Phalloidin (red) to mark actin filaments; and E- cadherin (green) as epithelial marker; nuclei counterstained with DAPI (blue)

Images courtesy of the Batlle Lab, IRB Barcelona, Spain

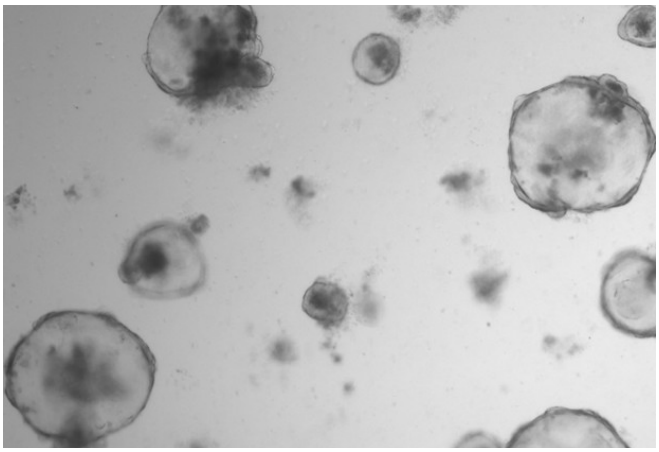


Human Colorectal Cancer (CRC) organoids grown for 7 days in ECM.

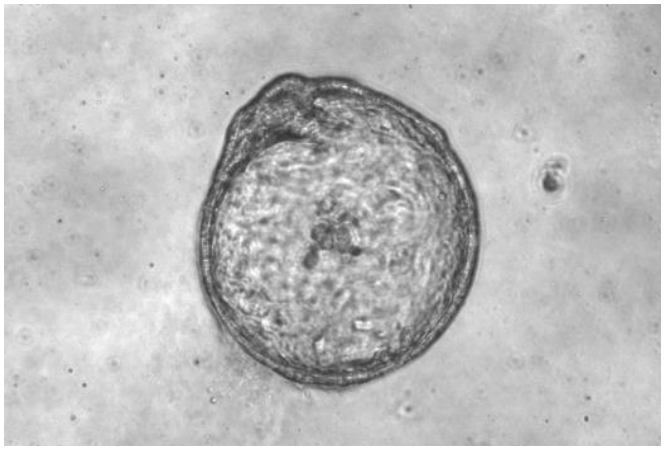
Brightfield (BF) and Immunofluorescence (IF) for Phalloidin (red) to mark actin filaments, and laminin (green), nuclei counterstained with DAPI (blue). Laminin stains basement membrane and mediates the attachment, migration and organization of cells.

Images courtesy of the Batlle Lab, IRB Barcelona, Spain

Transgenic Mouse



Organoids (passage 1) derived directly into ECM from AMSBIO. Organoids were split once (using mechanical disruption) following derivation; the image was taken 3 days later.



Existing organoid culture transferred to ECM. Organoids were digested to single cells, which were plated onto ECM. The image was taken 6 days after plating.

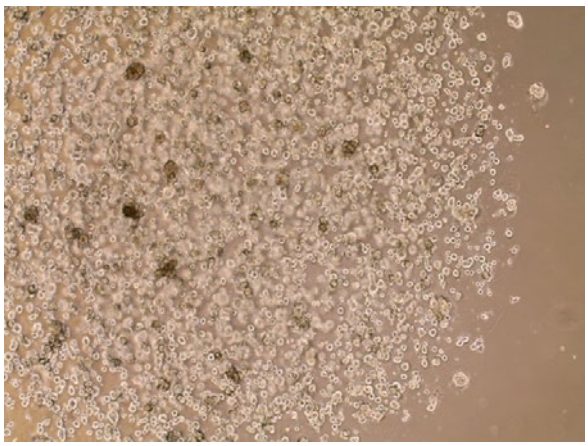
Organoids prepared from the intestinal crypts of transgenic mice.

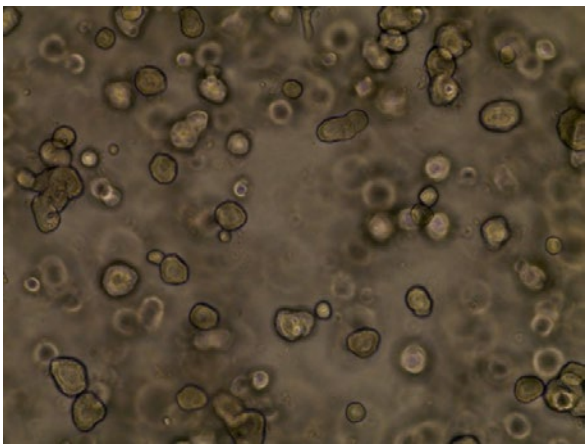
Images courtesy of the Sansom Lab, Beatson Institute of Cancer Research, Glasgow, UK

ESOPHAGEAL ORGANOID CULTURE

Esophageal Tumor

The images below show Organoids grown from tumors derived from fresh tissue samples. These images are showing cells taken from the lowest part of the esophagus almost into the stomach.

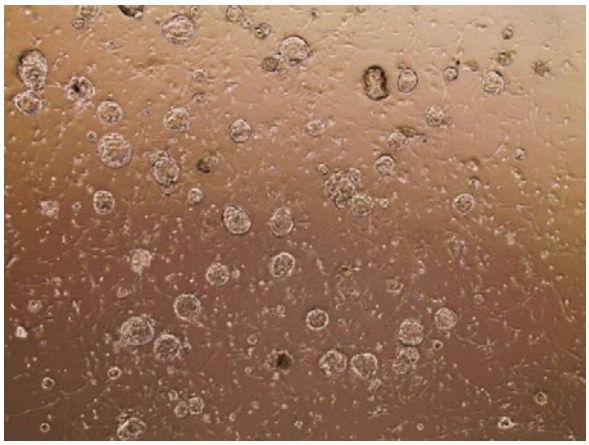


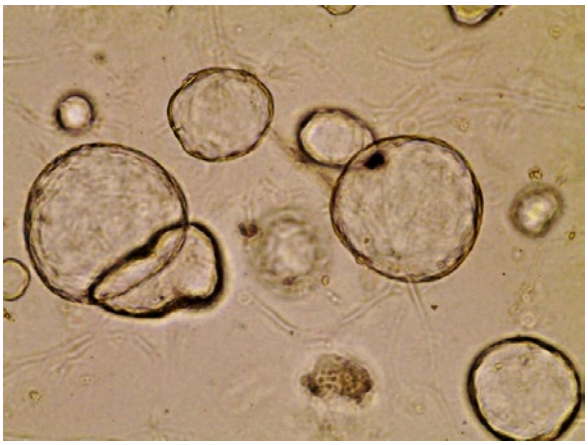


Images courtesy of Dr Mathew Garnett, Sanger Wellcome Trust Institute, Cambridge, UK

Esophageal Barrett's Epithelial

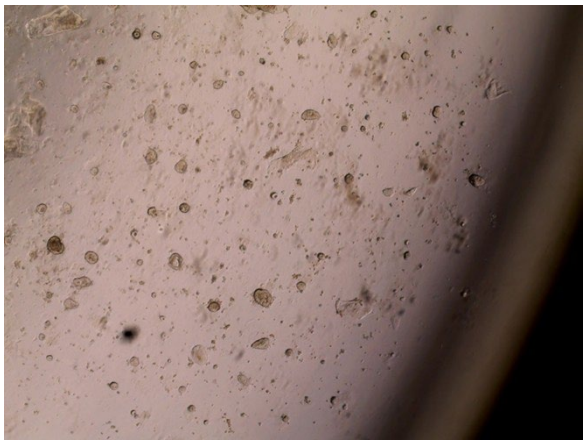
Barrett's esophagus is a precancerous condition where the esophageal lining becomes similar to the tissue architecture of the intestine, it can develop following long term cases of gastro-esophageal reflux disease (GERD).

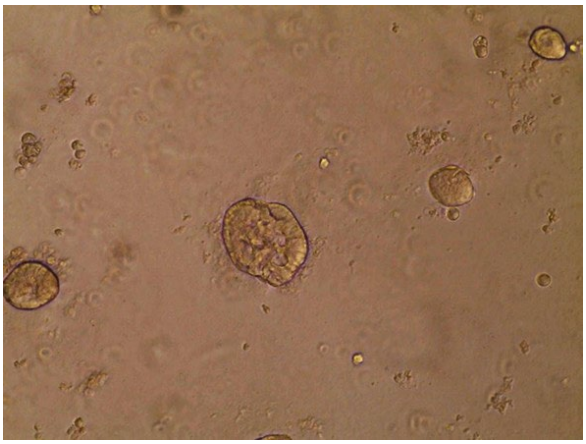




Images courtesy of Dr Mathew Garnett, Sanger Wellcome Trust Institute, Cambridge, UK

Esophageal Normal



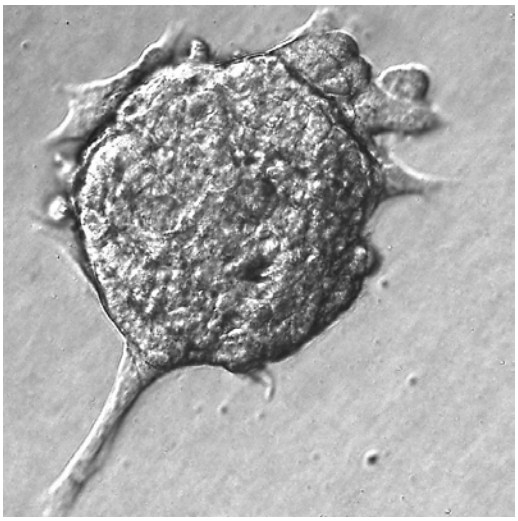


Images courtesy of Dr Mathew Garnett, Sanger Wellcome Trust Institute, Cambridge, UK

BREAST ORGANOID CULTURE

Mammary cancer organoid from mice, grown on / invading into ECM.

Image courtesy of Tuula Kallunki, Danish Cancer Society Research Centre, Copenhagen, Denmark

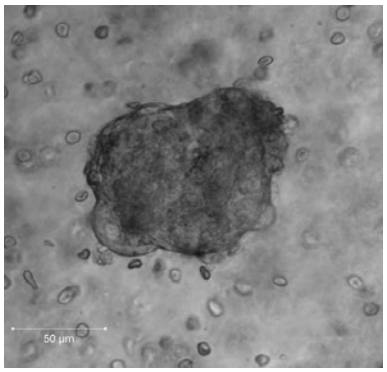


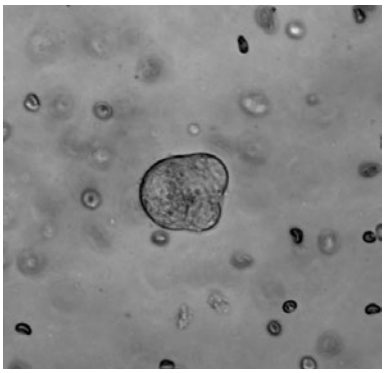
PROSTATE ORGANOID CULTURE

Metastatic Prostate Cancer-Derived Organoids

Confocal images of metastatic prostate cancer (mCRPC)-lymph node biopsy grown derived organoids on ECM from AMSBIO.

Images courtesy of Dr Veronica Gil, De Bono Lab, ICR, Sutton, UK





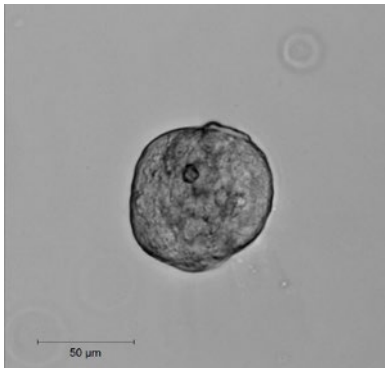
Harvested Organoid

This image on the right belong to same tumor biopsy-derived organoids from the pictures above and it was taken after first passage using Organoid Harvesting Solution and replating them.

The viability was very good and organoid proliferation rate has not been affected by the solution replating procedure, showing that the reagent is effective.

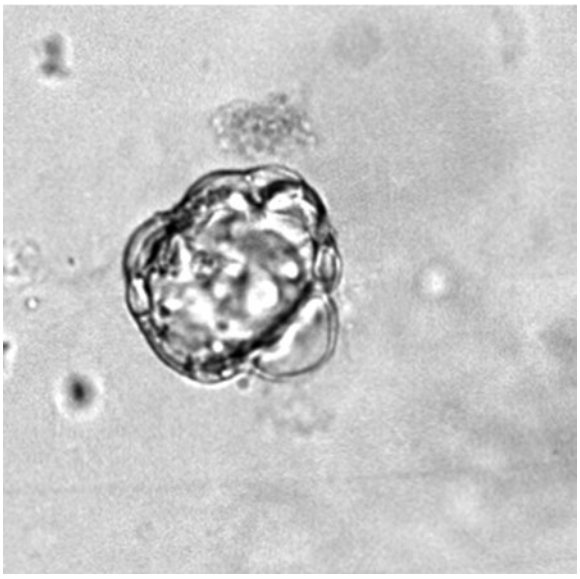
Image courtesy of Dr Veronica Gil, De Bono Lab, ICR, Sutton, UK

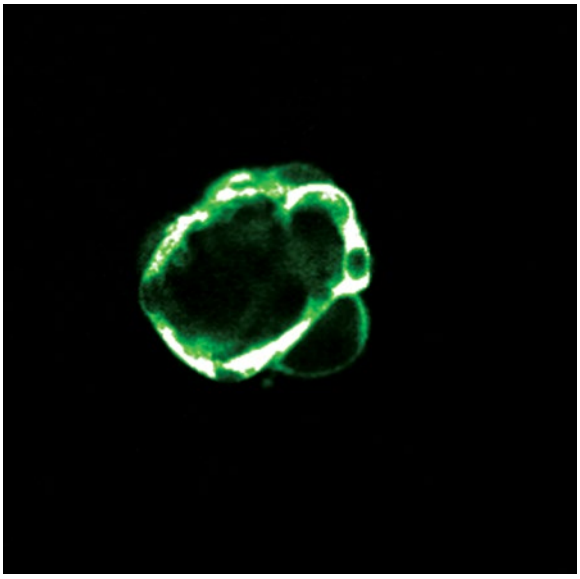
See page 22 for ordering details



Organoids from Mouse Prostate Stem Cells

Brightfield ***Alexa 488 (YFP)***





Images courtesy of the Baena lab, Prostate Oncobiology, CRUK Manchester Institute, Manchester, UK

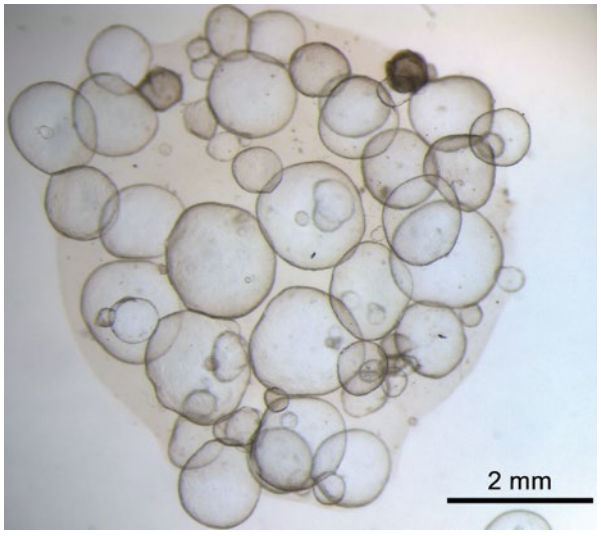
YFP-labeled organoids, grown from mouse prostate stem cells, grown on ECM.

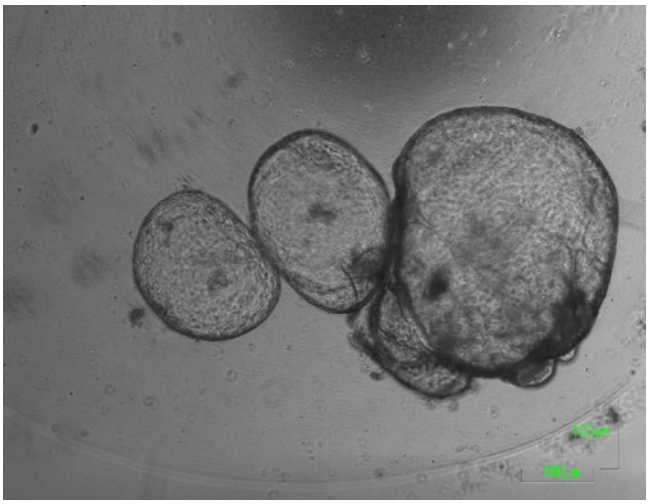
The prostate cells (from Rosa26 -LstopL-YFP mouse) were infected with Adenovirus expressing Cre to activate YFP expression

PANCREAS ORGANOID CULTURE

Human pancreatic organoids growing in ECM, 14 days after the isolation of ducts from the human pancreas.

Image courtesy of Dr. Meritxell Huch, Gurdon Institute, University of Cambridge





Fallopian Tube

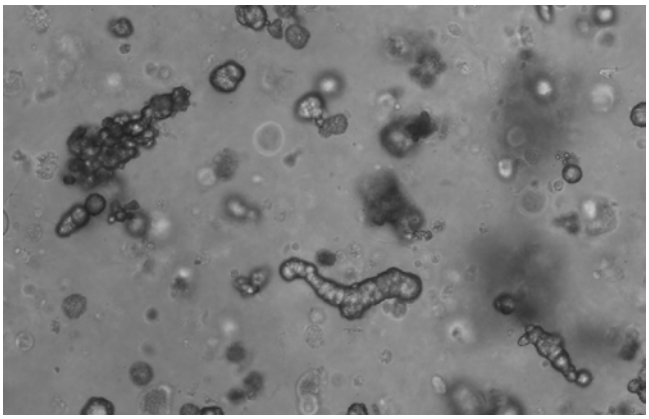
20 day culture of normal human fallopian tube organoids, grown in ECM.

Image courtesy of Dr Debbie Sanders, Brenton Lab, CRUK Cambridge Institute, Cambridge, UK

Ovary

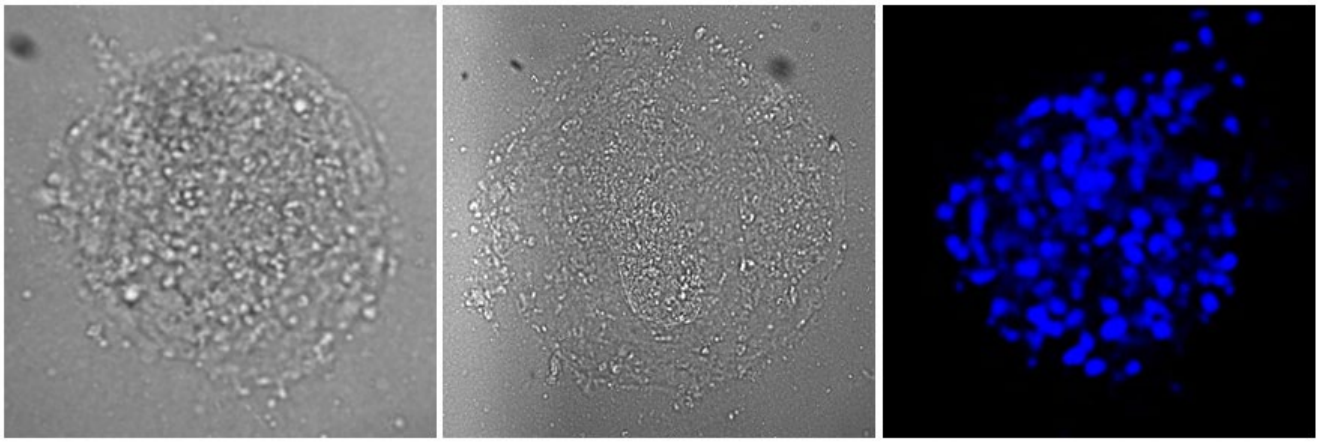
2 day culture of high grade serous ovarian cancer (HGSOC) organoids, grown in ECM.

Image courtesy of Dr Maria Vias, Brenton Lab, CRUK Cambridge Institute, Cambridge, UK



Uterus (Endometrium)

Bright field images (left and middle) and DAPI stained (right) image of the organoids isolated from Type 1 endometrial cancer patient specimens grown on ECM. Passage 2 Organoids were used in the study.



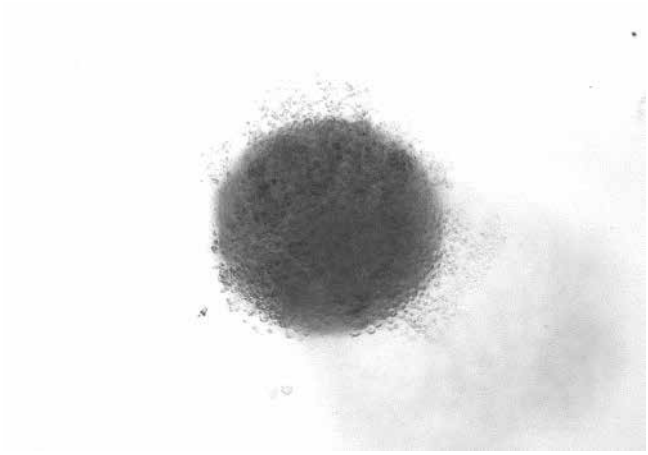
Data courtesy of Radhika Gogoi Lab, Weis Center for Research: from Oncotarget paper Verteporfin exhibits YAP-independent anti-proliferative and cytotoxic effects in endometrial cancer cells, reproduced under a Creative Commons Attribution 3.0 License

BRAIN ORGANOID CULTURE

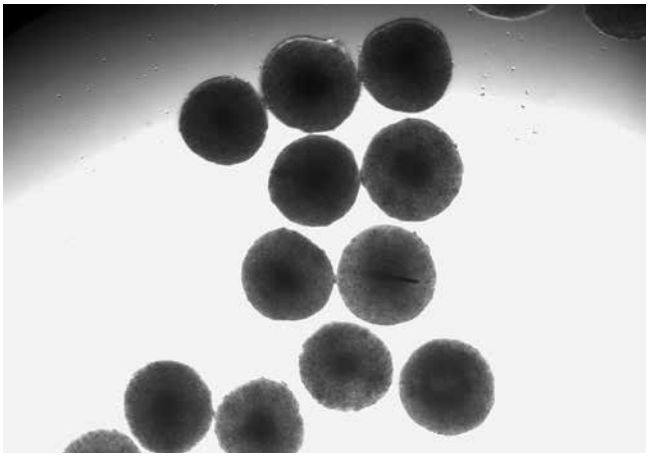
(A) Single iPSC-derived Embryoid Body (EB) generated in Lipidure®-Coat Plate A-U96 (96 well U-bottom plate).

(B) iPSC-derived EBs transferred from the Lipidure® Plate after a 6 day culture period in Lipidure®-Coat Plate A-U96 as above.

A



B



Images courtesy of Dr Julia Ladewig, Neural Development Group, Institute of Reconstructive Neurobiology, LIFE & BRAIN Center, University of Bonn, Germany

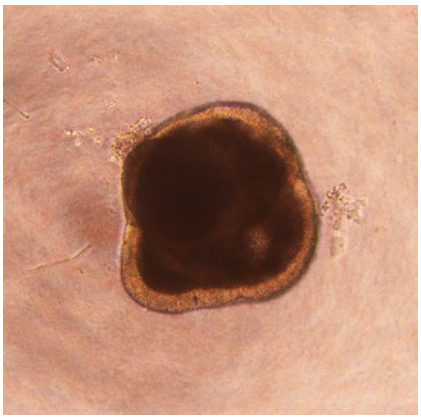
For Lipidure®-Coat plates ordering information see page 21

RETINA ORGANOID CULTURE

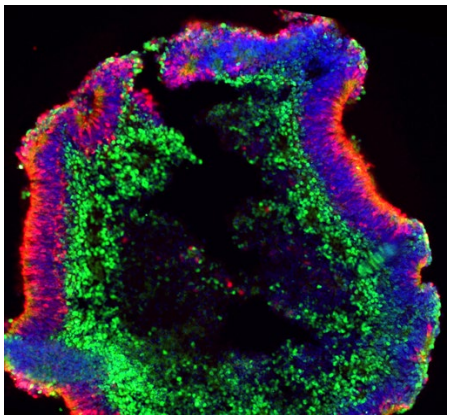
(A) Bright field image of retinal organoid taken at day 7 when the organoids are still in the Lipidure®-coated plate and eyefield induction has occurred.

(B) Immunohistochemistry evaluation of a mature day 21 retinal organoid section showing the layered structure of the organoid. The sample was stained for photoreceptor marker Recoverin (red), amacrine and ganglion cell marker Pax6 (green) and DAPI (blue).

Lipidure®-coated 96-well U-bottom

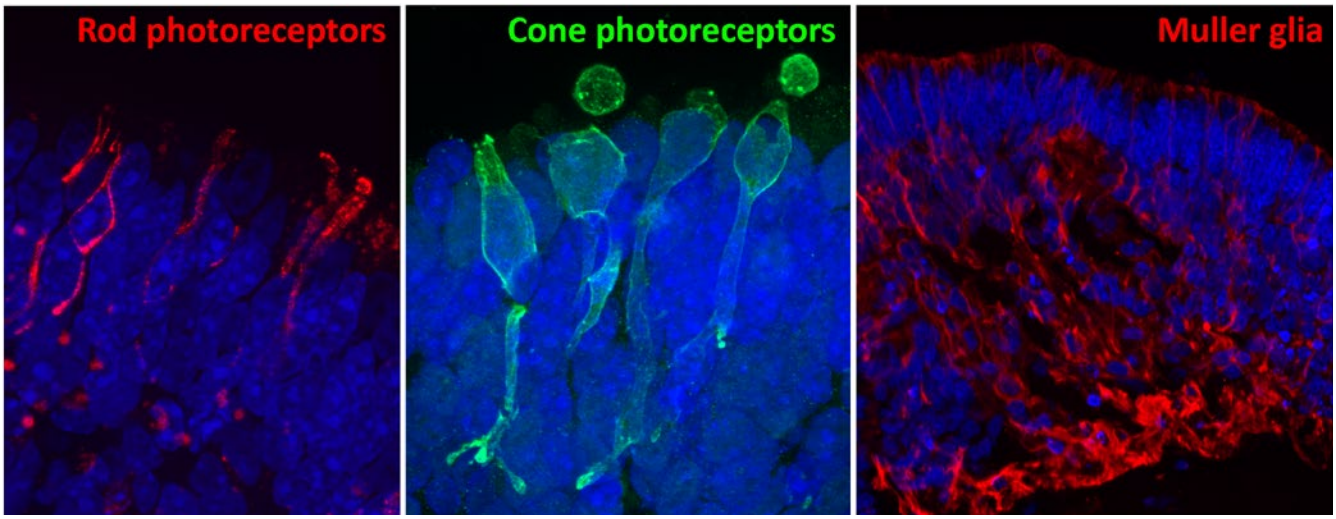


A



B

Images courtesy of Manuela Völkner and Dr. Mike Karl, German Center for Neurodegenerative Diseases (DZNE), Dresden, Germany



Images courtesy of Dr. Valeria Chichagova (Newcells Biotech) and Prof. Lako (Newcastle University)

Retinal organoids continuously grown on Lipidure®-coated plates from the start of differentiation. Immunofluorescence images of 150 day old organoids show rod and cone photoreceptors containing outer segments on the apical side and synaptic terminals at the base. Muller glial cells span across the entire thickness of retinal neuroepithelium.

"The Lipidure®-coated plates provided by AMSBIO were extremely useful for generating with ease large numbers of homogeneous retinal organoids which responded to light and contained all the key retinal cell types."

-Prof. Majlinda Lako, Newcastle University

For Lipidure®-Coat plates ordering information see page 21

Organoid Protocols

1. Sato, T., Vries, R. G., Snippert, H. J., van de Wetering, M., Barker, N., Stange, D. E., … & Clevers H. (2009). "Single Lgr5 stem cells build crypt–villus structures *in vitro* without a mesenchymal niche." *Nature* 459(7244), 262-265.

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3. a) Huch, M., Bonfanti, P., Boj, S. F., Sato, T., Loomans C. J., van de Wetering, M., Sojoodi, M., … & Clevers, H. (2013). Unlimited *in vitro* expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. *The EMBO Journal*, 32 (20), 2708-2721

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R-Spondin-1 Cell Line

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Lipidure®-coated 96-well U-bottom

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16. 13. Noel, G., Baetz, N. W., Staab, J. F., Donowitz, M., Kovbasnjuk, O., Pasetti, M. F., & Zachos, N. C. (2017). A primary human macrophage-enteroid co-culture model to investigate mucosal gut physiology and host-pathogen interactions. *Scientific Reports*, 7, 45270.

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CELLBANKER® 2 Cryopreservation Solution

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Front Cover images courtesy of Dr. Meritxell Huch, Gurdon Institute, University of Cambridge, UK; the Batlle lab, IRB Barcelona; and Helmuth Gehart/Professor Hans Clevers, Hubrecht Institute, Utrecht, Netherlands; Manuela Völkner and Dr. Mike Karl, German Center for Neurodegenerative Diseases (DZNE), Dresden, Germany

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