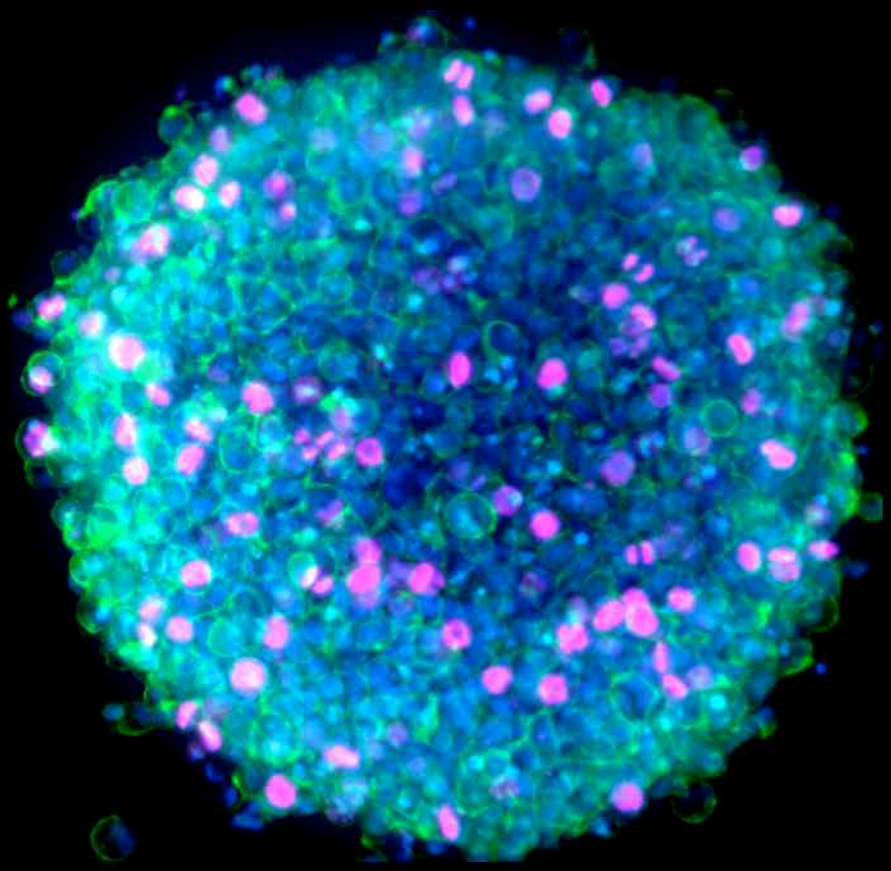


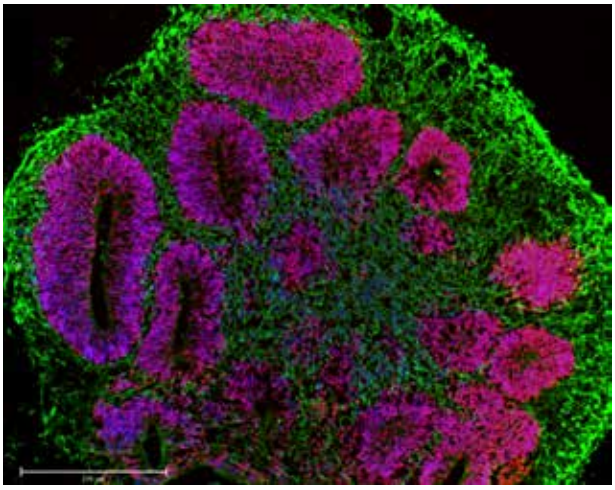
3D cell culture product selection guide for organoids and spheroids

ThermoFisher SCIENTIFIC

Introduction

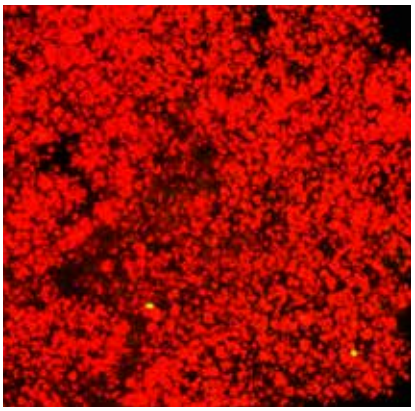
Organoids and spheroids show great potential in many applications, including drug discovery, toxicology, and disease modeling. These 3D cell models offer opportunities to better understand complex biology in a physiologically relevant context.

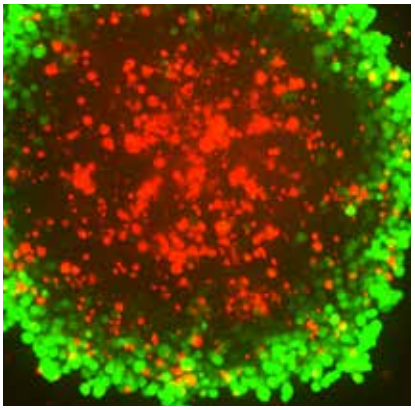
As advances in culturing organoids and spheroids become more common, the need for cell culture guidance and product recommendations is becoming more prevalent. This selection guide is intended to give researchers a helpful starting point to facilitate the transition from 2D monolayer cultures to 3D cell models.



Neural organoid cultured on a Thermo Scientific™ Nunclon™ Sphera™ 6-Well Plate, which allows cells to grow with virtually no attachment. The organoid was stained with antibodies conjugated to Invitrogen™ Alexa Fluor™ 488 and Alexa Fluor™ 594 dyes and imaged on an Invitrogen™ EVOS™ FL Auto 2 Imaging System at 10x magnification.

Contents



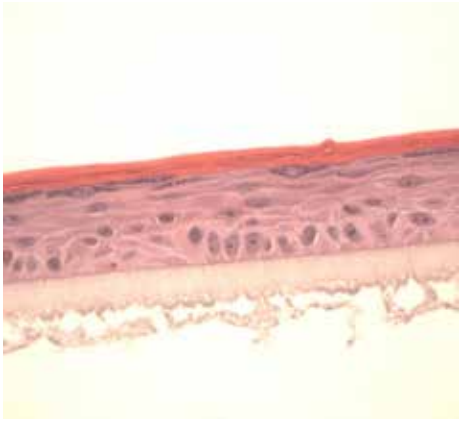


Cell sources

Physiologically relevant tissue models

Researchers utilize cell lines to investigate disease models of interest. Gibco™ cell lines allow you to closely mimic the *in vivo* state and generate more physiologically relevant data from organoid and spheroid cultures.

The table below shows a selection of our Gibco™ primary and stem cells.



Skin tissue model established from Gibco™ Human Epidermal Keratinocytes (HEKa) on Thermo Scientific™ Nunc™ Cell Culture Inserts and Carrier Plate System.

Ordering information

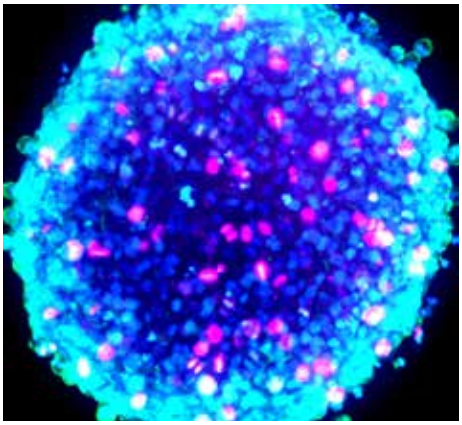
Product		Cat. No.
Primary cells: liver	Human Plateable Hepatocytes, Induction Qualified	HMCPIS
	Cryopreserved Human Kupffer Cells	HUKCCS
	Stellate Cells	Custom
	HepaRG Cells, Cryopreserved	HPRGC10
	Liver Sinusoidal Endothelial Cells	Custom
Primary cells: endothelial	Human Umbilical Vein Endothelial Cells (HUVEC)	C0035C
Primary cells: skin	Human Epidermal Keratinocytes, Neonatal (HEKn)	C0015C
	Human Epidermal Keratinocytes, Adult (HEKa)	C0055C
	Human Epidermal Melanocytes (HeMn), light, medium, or dark pigmentation	C0025C
		C1025C
		C2025C
	Human Dermal Fibroblasts, Neonatal (HDFn)	C0045C
	Human Dermal Fibroblasts, Adult (HDFa)	C0135C
	Human Microvascular Endothelial Cells, Neonatal Dermis (HMVECnd)	C0105C
Stem cells	Human Episomal iPSC Line	A18945
	Cas9 iPSC Line	Custom

Matrices and plasticware

Scaffold-based and scaffold-free offerings

When growing 3D cell cultures, the surface you choose is essential for reproducible results.

Scaffold-based systems are used to provide physical structures to support the assembly of cells into 3D models and to expand to significant numbers. Scaffold-free systems are matrix-free alternatives and are generally more adaptable to forming 3D cell models that are naturally established by endogenous adhesion molecules and extracellular matrices. Porous membrane–based systems are advantageous when polarization and differentiation of epithelial cells are needed in constructing 3D tissue models. Selecting the right culture platform is an important first step in developing a successful culture system for organoids and spheroids.



HeLa spheroid cultured in a scaffold-free Thermo Scientific™ Nunclon™ Sphera™ 96U-well plate.

Ordering information

Product		Cat. No.
Scaffold-based systems	Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix	A1413201
	AlgiMatrix 3D Culture System, 96-Well Plate	12684015
Scaffold-free systems	Nunclon Sphera Microplates, 96U-Well Plate	174925
	Nunclon Sphera Multidishes, 24-Well Plate	174930
	Nunclon Sphera Dishes, 35 mm Dish	174943
	Nalgene Single-Use PETG Erlenmeyer Flasks with Plain Bottom, 250 mL	41120250
Porous membrane–based and multilayer systems	Nunc Polycarbonate Cell Culture Inserts in Multi-Well Plates, 0.4 µm pore size, 24-well	140620
	Nunc 24-Well Carrier Plate with Cell Culture Inserts, 0.4 µm pore size	141002

Media systems to support growth

Growth, differentiation, and maturation of 3D cell models

The Gibco™ brand is the one most cited for media and reagents in peer-reviewed publications on organoid and spheroid research.* Gibco™ products are widely used and trusted for consistency in the growth, differentiation, and maturation of 3D cell models. Using the right combination of media and growth factors is vital to supporting the formation of disease-relevant 3D organoids and spheroids from specialized cell types like stem cells or cancer cell lines.

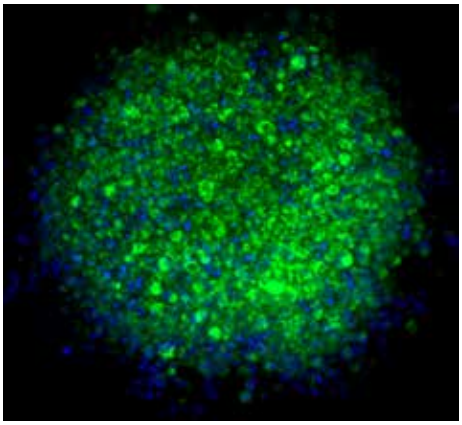
Ordering information

Product			Cat. No.
Pluripotent stem cells	Media	StemFlex Medium	A3349401
		Essential 8 Medium	A1517001
		Essential 8 Flex Medium Kit	A2858501
		StemPro hESC SFM	1000701
		Essential 6 Medium	A1516401
	Reagents	KnockOut Serum Replacement	10828010
		RevitaCell Supplement	A2644501
Skin cells	Media	Keratinocyte-SFM	17005042
		Medium 154	M154500
		EpiLife Medium	MEPI500CA
		Medium 254	M254500
		Medium 106	M106500
		MCDB 131 Medium	10372019
	Reagents	Human Keratinocyte Growth Supplement (HKGS)	S0015
		EpiLife Defined Growth Supplement (EDGS)	S0125
		Supplement S7	S0175
		Human Melanocyte Growth Supplement (HMGS)	S0025
		Low Serum Growth Supplement (LSGS)	S00310
		Microvascular Growth Supplement (MVGS)	S00525
Cancer cells and tumoroids	Media	DMEM/F-12, GlutaMAX Supplement	10565042
		Essential 8 Medium	11875093

* Based on a third-party market report covering papers cited for disease modeling of organoids and spheroids with primary or stem cells as the starting cell type.

Ordering information (cont.)

Product			Cat. No.
Hepatic cells	Media	Williams' E Medium	A1217601
		Advanced DMEM/F-12	12634010
	Reagents	HepExtend Supplement	A2737501
		Primary Hepatocyte Maintenance Supplements	CM4000
Brain cells	Media	Neurobasal Medium	21103049
	Reagents	B-27 Supplement, serum free	17504044
		B-27 Supplement, minus insulin	A1895601
		B-27 Supplement, minus vitamin A	12587010
		N-2 Supplement	17502001
Multiple cell types	Media	DMEM/F-12, GlutaMAX Supplement	10565042
		RPMI 1640 Medium	11875093

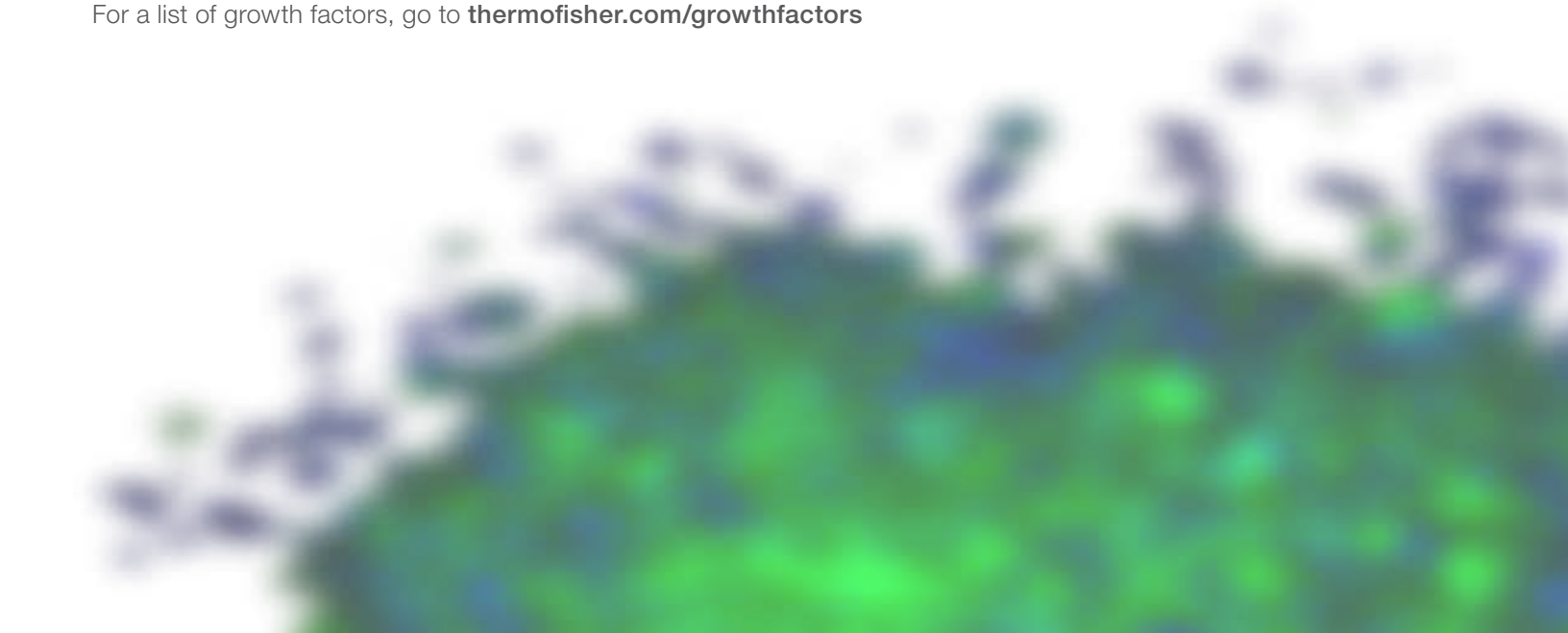


A549 cells were plated at a density of 5,000 cells/well on a Nunclon Sphera U-bottom plate and incubated for 24 hr in a CO_2 incubator. The spheroids were then stained with Invitrogen™ Image-iT™ Green Hypoxia Reagent at a final concentration of 5 µM and Hoechst 33342 for 1 hr. The plate was imaged with a 10x objective using confocal mode on a Thermo Scientific™ CellInsight™ CX7 LZR High Content Analysis Platform. The image is from a maximum-intensity projection of 20 optical Z slices of 10 µm each.

Growth factors

High-quality Gibco™ growth factors are designed to give you high biological activity, high purity (95% pure), and <0.1 ng endotoxin per microgram. Our growth factors are verified with Gibco media for proven compatibility.

For a list of growth factors, go to **thermofisher.com/growthfactors**



Tools for monitoring and detection

Gene expression

qPCR

When, where, and under what conditions are genes expressed? What triggers, or prevents, this expression? Scientists are discovering the surprisingly wide range of transcription and translation products, and how these different expression products determine the growth and health of an organism.

TaqMan Assays

Over 1.8 million predesigned Applied Biosystems™ TaqMan® Gene Expression Assays covering a growing list of model species have been predesigned using long-standing bioinformatics expertise in primer and probe design.

For more details, go to **thermofisher.com/taqman**

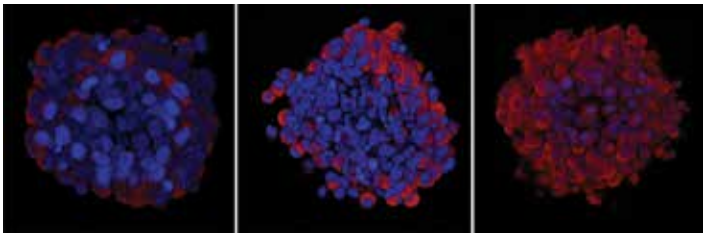
Ordering information

Product		Cat. No.
QuantStudio real-time PCR systems	QuantStudio 3 Real-Time PCR System, 96-well, 0.2 mL	A28137
	QuantStudio 5 Real-Time PCR System, 96-well, 0.2 mL	A28139
	QuantStudio 6 Flex Real-Time PCR System, 96-well, laptop	4485689
	QuantStudio 7 Flex Real-Time PCR System, array card, laptop	4485700
	QuantStudio 12K Flex Real-Time PCR System, Fast 96-well block, desktop	4471088

Cell health

Reagents for 3D models

Ensuring cells are maintaining the appropriate physiological morphology, markers, and activity is paramount to ensuring successful research outcomes. We have a full line of plate readers, imaging systems, and high-content analysis platforms to help you image and analyze your spheroids and organoids. These easy-to-use systems, combined with our broad portfolio of Invitrogen™ fluorescent reagents for cellular assays, allow researchers to effectively evaluate and understand 3D cell models.



Spheroid staining using Invitrogen™ CellROX™ Deep Red Reagent. HeLa spheroids were pretreated with 100 µM menadione. Cells showing oxidative stress are stained red, and live-cell nuclei are stained blue.

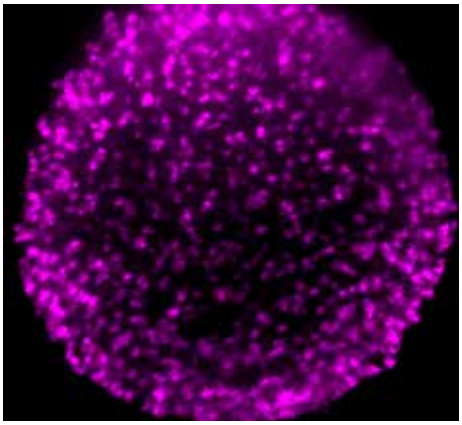
Ordering information

Product		Cat. No.
Cell health reagents	Image-iT Fixation/Permeabilization Kit	R37602
	NucBlue Live ReadyProbes Reagent	R37605
	Click-iT Plus EdU Alexa Fluor 488 Imaging Kit	C10337
	Image-iT Red Hypoxia Reagent	H10498
	Image-iT Green Hypoxia Reagent	I14834
	CellEvent Caspase-3/7 Green Detection Reagent	C10723
	LIVE/DEAD Viability/Cytotoxicity Kit	L3224
	CellTracker Deep Red Dye	C34565
	CellROX Deep Red Reagent	C10422
	MitoTracker Orange	M7510
	ProLong Glass Antifade Mountant	P36980
	LysoTracker Deep Red	L12492
	Calcein, AM	C3100MP

Tools for imaging and analysis

Visualization of 3D cell models

Growing 3D models is a large investment in time and resources, and you need reassurance that your investment is going to give you the 3D models that you anticipate. Our visualization tools allow you to monitor the formation of your organoids and 3D models to give you confidence that you are heading in the right direction. These imaging and high-content analysis platforms have been recognized as trustworthy systems for analyzing organoid and spheroid cultures. In addition, Invitrogen™ antibodies are validated* to help ensure specificity and reproducibility in research results.



HeLa cells were plated at a density of 5,000 cells/well on a Nunclon Sphera U-bottom plate and incubated for 24 hr in a CO_2 incubator. The spheroids were then fixed with 4% formaldehyde and permeabilized with 0.25% Triton™ X-100. The spheroids were blocked with 3% BSA and then stained with Ki-67 antibody conjugated to Alexa Fluor 647 dye using the Invitrogen™ Zip Alexa Fluor™ 647 Rapid Antibody Labeling Kit. The plate was imaged with a 10x objective using confocal mode on a CellInsight CX7 LZR High Content Analysis Platform. The image is from a maximum-intensity projection of 20 optical Z slices of 10 µm each.

Ordering information

Product		Cat. No.
Instruments	Varioskan LUX Multimode Microplate Reader	VLB000D0
	EVOS FL Auto 2 Imaging System	AMAFD2000
	CellInsight CX7 LZR High Content Analysis Platform	CX7A1110LZR
	EVOS XL Core Imaging System	AMEX1000
	EVOS XL Core Attachable Mechanical Stage	AMEP4712

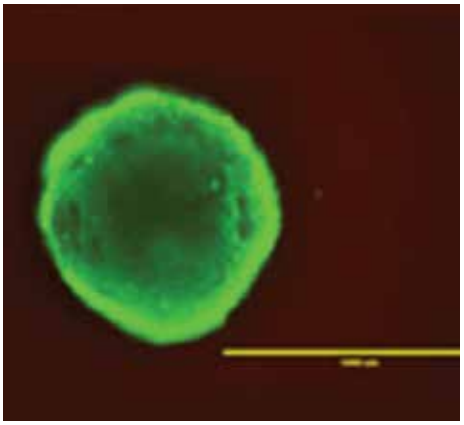
* The use or any variation of the word “validation” refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic use.

Key protocols and methods

Most-cited publications

There is a wide range of protocols and methods for 3D cell model formation published to date. Table 1 is a selection of the seminal publications for different cell types, to help you get started on your journey to formation of 3D cell models.

HCT 116 (1,000 cells/well)



Spheroid cell viability assay. Spheroid cell viability was evaluated using the Invitrogen™ LIVE/DEAD™ Cell Imaging Kit, where live cells are stained green and dead cells are stained red. Scale bar = 1,000 µm.

Table 1. Publications for 3D cell models.

Organ	Model type	Cell type	Differentiated cell type	Relevant growth factor	Relevant medium	Key publication
Brain	Organoid	Pluripotent stem cells	• Ventricular zone radial glial cells • Cortical neurons	NA	DMEM/F-12, Neurobasal	Iefremova V et al. (2017) *Cell Reports* 19:50-59
			• Cortical progenitor cells • Cortical neurons	BDNF, GDNF, NT-3, laminin, bFGF	DMEM/F-12, Essential 8, Essential 6, Neurobasal	Zhou T et al. (2017) *Cell Stem Cell* 21:274-283
			• Cortical neurons	DKK1	DMEM/F-12	Phillips AW et al. (2017) *J Vis Exp* 125:e55799.
			• Cortical neurons • Astrocytes	FGF2, EGF, BDNF, NT-3	DMEM/F-12, Neurobasal	Pasca AM et al. (2015) *Nature Methods* 12:671-678
			• Cortical neurons • Radial glial stem cells • Retinal cells	FGF2	DMEM/F-12, Neurobasal	Lancaster MA et al. (2013) *Nature* 501:373-379
			• Cortical neurons	DKK1, BMPR1a-Fc	DMEM/F-12, Neurobasal	Mariani J et al. (2012) *Proc Natl Acad Sci U S A* 109:12770-12775
Liver	Spheroid	Primary cells	• Hepatocytes	NA	HepaRG	Proctor WR et al. (2017) *Arch Toxicol* 91:2849-2863
	Organoid	Pluripotent stem cells	• Hepatocytes • Septum transversum mesenchyme • Endothelial cells	BMP4, VEGF, activin, PDGF-BB, FGF2	RPMI 1640, DMEM/F-12, StemPro-34 SFM	Takebe T et al. (2017) *Cell Reports* 21:2661-2670
			• Hepatocytes • Cholangiocytes	Activin, BMP4, FGF2, FGF10, EGF, HGF	DMEM/F-12, RPMI 1640	Guan Y et al. (2017) *JCI Insight* 2:e94954
			• Hepatocytes	Activin, TGFbeta, FGF2, BMP4, FGF4, DKK1, OSM, HGF	IMDM, F-12	Pettinato G et al. (2016) *Sci Rep* 6:32888
		Adult stem cells	• Hepatocytes	EGF, FGF10, HGF, noggin, BMP7, FGF19	Advanced DMEM/F-12	Huch M et al. (2015) *Cell* 160:299-312
		Adult stem cells	• Hepatocytes	FGF10, HGF, EGF, noggin	Advanced DMEM/F-12	Huch M et al. (2013) *Nature* 494:247-250
		Pluripotent stem cells	• Hepatocytes • HUVECs • Mesenchymal stem cells	FGF2, BMP4, activin, HGF, EGF	RPMI 1640	Takebe T et al. (2013) *Nature* 499:481-484

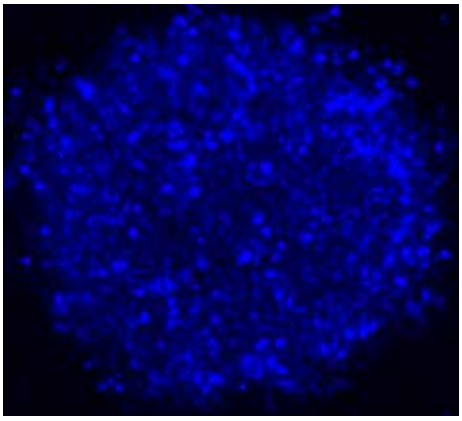
Table 1. Publications for 3D cell models. (cont.)

Organ	Model type	Cell type	Differentiated cell type	Relevant growth factor	Relevant medium	Key publication
Lung	Organoid	Pluripotent stem cells, fetal cells	• Fibroblasts • Mesenchymal stem cells • HUVECs • Type I alveolar cells • Type II alveolar cells	FGF2	DMEM/F-12	Wilkinson DC et al. (2017) *Stem Cells Transl Med* 6:622-633
		Pluripotent stem cells	• Basal cells • Ciliated cells • Club cells • Mesenchymal stem cells • Alveolar progenitor cells	Activin, FGF4, FGF2, noggin, SHH	RPMI 1640, Advanced DMEM/F-12	Dye BR et al. (2015) *eLife* 4:e05098
Intestine	Organoid	Pluripotent stem cells	• Goblet cells • Enteroendocrine cells • Mesenchymal stem cells	Activin, FGF4, BMP2, EGF, noggin, SHH	Advanced DMEM/F-12, RPMI 1640	Munera JO et al. (2017) *Cell Stem Cell* 21:51-64
			• Enterocytes • Paneth cells • Goblet cells • Enteroendocrine cells • Innervated smooth muscle cells	IGF1, FGF2	KnockOut DMEM	Uchida H et al. (2017) *JCI Insight* 2:e86492
			• Enterocytes • Paneth cells • Goblet cells • Enteroendocrine cells • Tuft cells • Smooth muscle cells	Activin, FGF4, EGF, noggin	RPMI 1640, Advanced DMEM/F-12	Watson CL et al. (2014) *Nat Med* 20:1310-1314
			• Enterocytes • Paneth cells • Goblet cells • Enteroendocrine cells • Mesenchymal stem cells	Activin, FGF4, noggin, EGF	DMEM/F-12, RPMI 1640, Advanced DMEM/F-12	Spence JR et al. (2011) *Nature* 470:105-109
		Adult stem cells	• Enterocytes • Paneth cells • Goblet cells • Enteroendocrine cells	Noggin, EGF, FGF10	DMEM, Advanced DMEM/F-12	Sato T et al. (2011) *Gastroenterology* 141:1792-1772
			• Enterocytes • Paneth cells • Goblet cells • Enteroendocrine cells	EGF, noggin	Advanced DMEM/F-12	Sato T et al. (2009) *Nature* 459:262-265
Kidney	Organoid	Fetal tissue–derived cells	• Nephron progenitor cells • Glomerular cells • Proximal tubule epithelial cells • Loop of Henle epithelial cells • Distal tubule epithelial cells	BMP7, FGF2, LIF	DMEM/F-12	Li Z et al. (2016) *Cell Stem Cell* 19:516-529
		Pluripotent stem cells	• Proximal tubule epithelial cells • Loop of Henle epithelial cells • Distal tubule epithelial cells • Podocytes	FGF2, noggin, activin, FGF9	Advanced RPMI 1640	Morizane R et al. (2015) *Nat Biotechnol* 33:1193-1200
		Pluripotent stem cells	• Metanephric mesenchyme • Ureteric epithelial cells	FGF2, BMP4, activin, BMP7, FGF9	DMEM/F-12	Takasato M et al. (2015) *Nature* 526:564-568

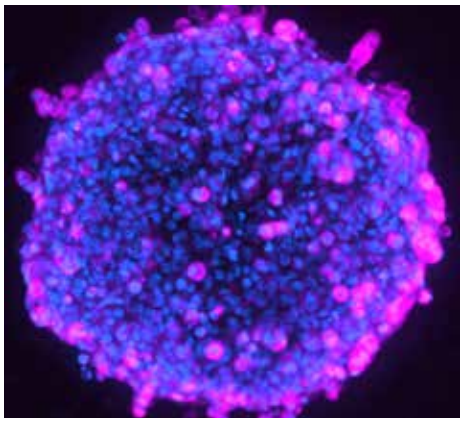
Custom biology solutions

Extend your research capabilities and partner with our custom biology team

Scientists today are continually being asked to transition their research to more physiologically relevant disease models. Whether your team needs help developing the right cell model or is interested in outsourcing steps of the research project, our CellModel Services team can help provide a custom 3D cell model tailored to your research needs. Reach out to us at **thermofisher.com/cellmodels**.



A549 cells were plated at a density of 5,000 cells/well on a Nunclon Sphera U-bottom plate and incubated for 24 hr in a CO_2 incubator. The spheroids were then stained with Hoechst 33342 for 1 hr. The plate was imaged with a 10x objective using confocal mode on a CellInsight CX7 LZR High Content Analysis Platform. The image is from a maximum-intensity projection of 20 optical Z slices of 10 µm each.



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Find out more at **thermofisher.com/3dmodel**

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