

## **3D CELL CULTURE SOLUTIONS**

For more representative results

Because biology lives in a 3-dimensional world.





# MIMICS IN VIVO PHYSIOLOGICAL CONDITIONS

by replicating native tissue microenvironment



# IN VITRO WITH IN VIVO CELL-CELL INTERACTIONS

by promoting cell-cell contact and interaction with ECM for predictive drug response



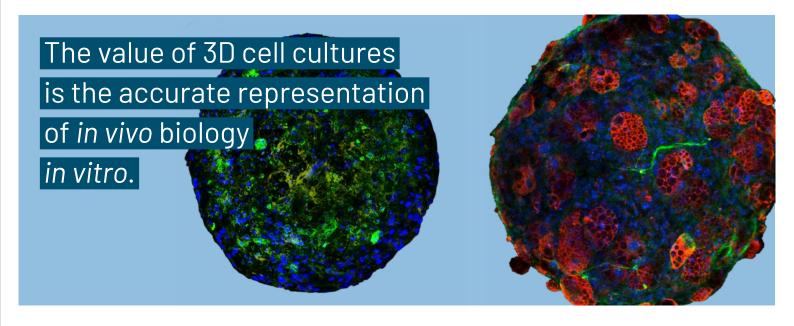
# CO-CULTURE OF SEVERAL CELL TYPES

and possibility of human cell usage



## **NO ETHICAL DIFFICULTIES**

compared to animal usage - Replacement, Reduction and Refinement (3Rs)



## Open your mind to 3D cell culture. Go deeper - and find out what's really there

Not long ago, researchers were sceptical of the value of culturing cells in a 3D environment. Today, 3D cell culture has become a routine tool that plays a vital role in life sciences, especially in cancer research, drug discovery and toxicology. 3D cell culture approaches are still relatively new despite their early impact. There is still ambiguity in the robustness of culturing techniques, the readiness of the supply chain, and even the use of terminology depicting 3D morphology. The value of 3D cell cultures is defined by the increased dimensionality and access to contact between cells to generate a phenotype predictive of in vivo biology but performed in vitro.

Cell-cell interaction is fundamental, independent of whether 3D structures are called spheroids, organoids, assembloids, or tumoroids, and of course, a phenotype is agnostic to terminology.

Greiner Bio-One has three main product options available to perform 3D cell culture to give the best solution for every specific research question. ThinCert® cell culture inserts to mimic a variety of *in vivo* situations by the two-compartment system.

CELLSTAR® cell culture vessels with cell-repellent surfaces prevent cell adherence to promote the spontaneous formation of three-dimensional spheroids and as an essential accessory for the magnetic 3D cell culture technology (m3D). M3D creates 3D cell culture structures by magnetizing cells and using magnet forces.

# THE VALUE OF GREINER BIO-ONE'S 3D CELL CULTURE TECHNOLOGIES:

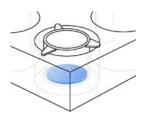
- / Easy to mimic
   accurate
  representation
  of native tissue
  environment
- / Easy to overcome drawbacks leave behind disadvantages of 2D cell culture and animal experiments for more relevant cell models
- / Easy to scale scalable systems
  to increase the
  throughput
- Easy to setup

   confirmed by
   independent
   scientific
   publications

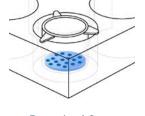
## 3D CELL CULTURE PRODUCT PORTFOLIO **OVERVIEW**

#### ThinCert® Cell Culture Inserts

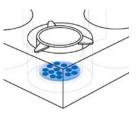
Format: available in 6, 12 and 24 well format



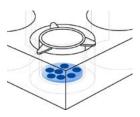
Pore size 0.4 µm



Pore size 1.0 µm



Pore size 3.0 µm



Pore size 8.0 µm

- Pore density 2 x 10<sup>6</sup>/cm<sup>2</sup> / [transparent]
- Pore density 1 x 108/cm2 [translucent]
- Pore density 2 x 10<sup>6</sup>/cm<sup>2</sup> [transparent]
- Pore density 0.6 x 10<sup>6</sup>/cm<sup>2</sup> / [transparent]
- Pore density 1x10<sup>6</sup>/cm<sup>2</sup> [translucent]
- Pore density 0.15 x 108/cm2 [translucent]

#### CELLSTAR® Cell-Repellent Vessels

Raw material: Polystyrene



Dishes

35, 60 and 100 mm

Flasks



Flat bottom plates



- diameter
- With vents
- 50, 250, 550 and 650 ml version
- With screw or filter screw cap

- 96, 384 and 1536 well version
- Clear, black and white with µclear bottom

U-bottom plates

- 96 and 384 well version
- Clear

#### Magnetic 3D Cell Culture Solutions

Format: available for different well formats



**Bioprinting Kits** 

96, 384 and 1536 well



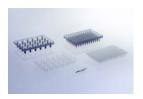
Bio-Assembler Kits

6 and 24 well



BioAssay Kits

96 and 384 well



MagPen Kits

Single, 24 and 96 well Multi-MagPen

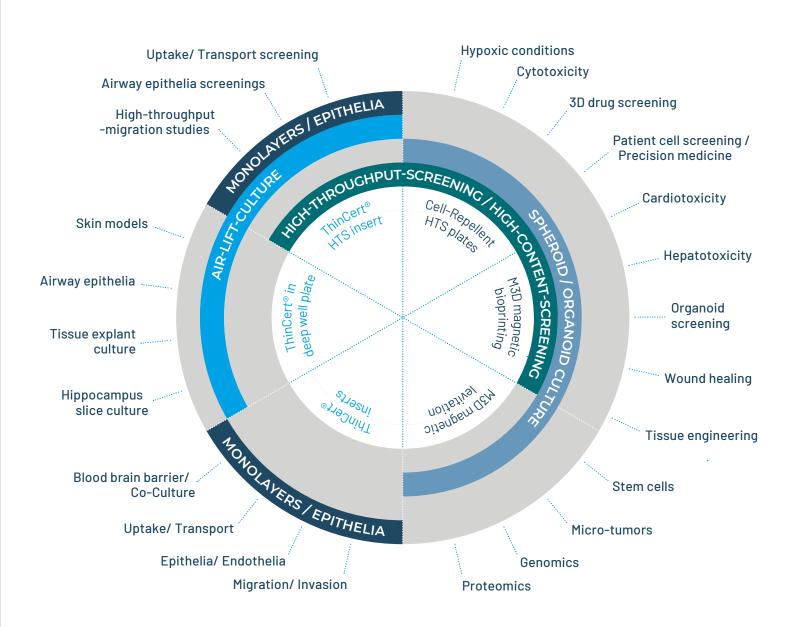
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# MATCHING PRODUCTS FOR VARIOUS APPLICATION FIELDS

The application fields for 3D cell culture are very diverse and wide-ranging, from migration assays over tissue engineering to high-throughput drug discovery and toxicology studies 3D cell culture is an essential tool. Depending on the specific application and scientific asked question independent if in basic research or in the industrially environment the right product either in low- or in high-throughput wants to be selected.

Greiner Bio-One offers a wide range of solutions for all kind of 3D cell culture applications - let's overcome limits together!



# THINCERT® CELL CULTURE INSERTS

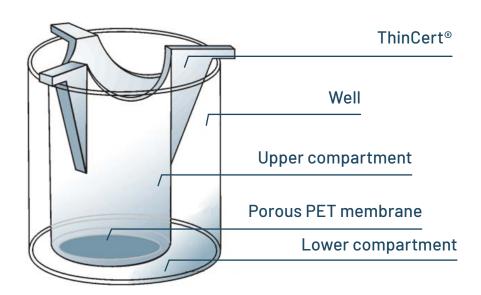
# BROAD PRODUCT RANGE FOR VARIOUS CELL CULTURE ASSAY APPLICATIONS

For advanced cell and tissue culture applications, Greiner Bio-One offers an extensive family of membrane supports - ThinCert®. Combining 6 different membrane specifications (pore size and density) in geometries to fit 6, 12 and 24 well plates, the ThinCert®

cell culture inserts are suitable for a wide range of applications including transport, secretion and diffusion studies, migrational experiments, cytotoxicity testing, co-cultures, trans epithelial electric resistance (TEER) measurements and primary cell culture.

#### THINCERT® - THE PRINCIPLE

- / ThinCert® with porous PET membrane in different pore sizes
- / PET capillary pore membrane thermobonded onto insert
- / Insert and well form a two-compartment system
- / Compartments can communicate via membrane pores
- / Improved cell adhesion through physical surface treatment
- / Enhanced pipetting access and gas exchange





#### Migration/ Invasion assay

Cell migration plays a significant role in physiological and pathological processes during embryonic development, wound healing, immune response, inflammation, and tumorigenesis. The filter assay is a standard in vitro model used to study cell migration and is performed in cell culture inserts with 8.0 µm pores of the ThinCert® membrane, which supports and involves cell migration from the upper compartment towards a chemo-attractant source in the lower compartment.

# MAIN REQUIREMENTS DERIVED FROM APPLICATION

ThinCert® cell culture inserts with porous PET membranes thermobonded to a clear polystyrene housing form a two-compartment system to mimic a variety of *in vivo* situations. The inserts entail a design with an eccentric position in the wells for facilitating pipette access to the lower compartment. The sterility of the single blister-packed inserts and multiwell plates is ensured by irradiation.

Greiner Bio-One offers several application protocols for the practical integration of ThinCert® cell culture inserts addressing a specific question and provideing detailed laboratory instructions.



#### Co-Culture

Co-culture includes diverse applications, such as the study of immune cell interactions, the stimulation of cell proliferation, the maintenance of cell differentiation, and the restoration of heterocellular functions in vitro (e.g. bloodbrain-barrier). With ThinCert® cell culture inserts, cells may be seeded in the upper and lower compartment so the  $0.4 \, \mu m$  or  $1.0 \, \mu m$  pores of the ThinCert® membrane allow the exchange of molecules between the two cell populations.



#### Transport Studies

Transport studies are among the most frequent applications of cell culture inserts. The goal is to reconstruct a functional epithelium from individual cells and study the active transport of substances from one compartment through the epithelium to the other compartment. ThinCert® cell culture inserts with 0.4 µm pores and translucent membranes are recommended for transport assays.



#### Organotypic and air-liftculture

In organotypic culture a tissue can be kept alive for prolonged periods, while in tissue reconstruction the tissue is de novo generated from single cells. Both procedures use cell culture inserts with 0.4 to 3.0  $\mu m$  pores and entail tissue growth in vitro at the air-liquid-interface without limitations from gas exchange. Furthermore, for some tissue types, the direct exposure of the cultivated cells to the surrounding atmosphere serves as an indispensable differentiation stimulus.



# CELLSTAR® CELL-REPELLENT VESSELS

# PREVENT CELL ATTACHMENT TO SUPPORT THE FORMATION OF 3D CELL CULTURES

In contrast to standard tissue culture surfaces optimized to enhance cell attachment conditions, the CELLSTAR® cell-repellent surface has been developed to prevent cell attachment effectively. For the formation of e.g. spheroids or self-assembled spherical clusters used as 3D cell culture models, the cell-cell interac-

tion must dominate over the interaction between the cells and the culture surface of containment.

CELLSTAR® cell-repellent products are by default sterile, non-cytotoxic, free of detectable endotoxins, detectable DNase / RNase and human DNA and have a 4 year shelf-life.

#### **APPLICATIONS**

- / Suspension culture of semi-adherent and adherent cell lines
- / Spheroid culture of a single spheroid per well in round-bottom, or multiple spheroids in flat-bottom microplates, flasks and dishes
- Aggregation of stem cells, as a key step for cultivation and differentiation in 3D
- Joulture in hydrogels without cell migration out of the hydrogel and formation of 2D subcultures
- / Indispensable part of the magnetic 3D cell culture technology



#### **GO DEEPER!**

Find detailed information in the related FORUM NO. 17 at our Download Center.



# MAGNETIC 3D CELL CULTURE SOLUTIONS

#### BECAUSE 3D MUST NOT BE COMPLICATED

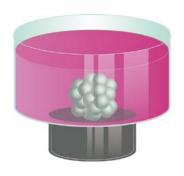
The core technology of Greiner Bio-One's Magnetic 3D Cell Culture is the magnetisation of cells with NanoShuttle-PL. The magnetised cells can be aggregated by magnetic forces, either by levitation or bioprinting, to form microphysiologically relevant 3D models *in vitro*. The magnetic system overcomes the limitations of other platforms by enabling the manipulation of cells for the rapid formation of spheroids, repro-

ducible and scalable in size for high-throughput, without restrictions of cell type. When used in tandem with commercially available standardised biochemical assay methods, the magnetic 3D cell culture method facilitates continuous assessment of cell viability and other functions to provide an ideal platform for e.g. compound or viability screenings or genomics or protein analysis.



#### Levitation 6 and 24 well

By levitating cells from the bottom of a cell-repellent plate, magnetic forces work as an invisible scaffold to gently and rapidly aggregate cells, forming larger spheroids at the air-liquid interface, induce cell-cell interactions and initiate ECM synthesis.



#### Bioprinting 96, 384 and 1536 well

With bioprinting magnetised cells are printed into spheroids by placing a cell-repellent plate atop a drive of magnets, where a single magnet below each well utilises mild magnetic forces to induce aggregation and print one spheroid at the bottom of each well within 15 minutes to a few hours.

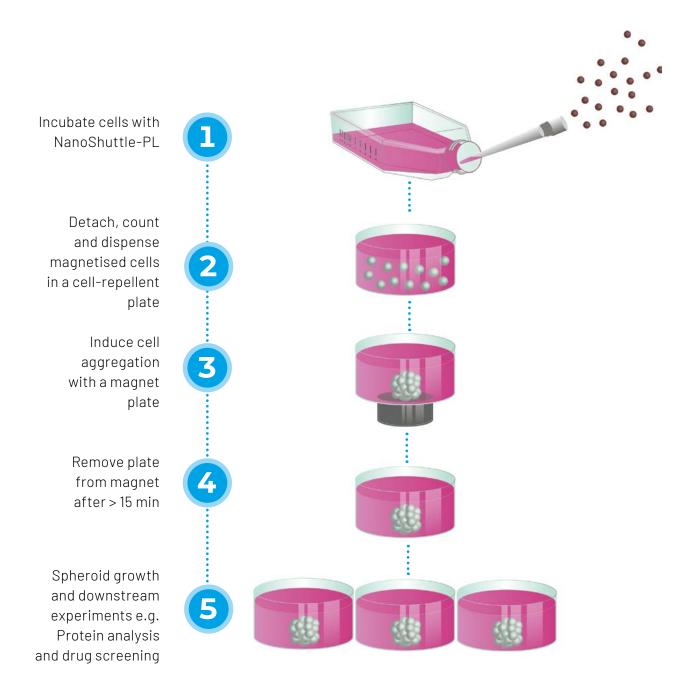


#### Bio Assay 96 and 384 well

In addition bioprinting with a spheroidal shape, magnetic printing of cells can also be patterned into a ring formation. For up to 72 hours immediately following bioprinting, the patterned structures will shrink/close as a function of cell migration, viability, cell-cell interaction, and/or proliferation.

Find this and further information on our website

# 3D CELL CULTURE FORMATION IN A FAST AND EASY WORKFLOW!



# MAGPEN - YOUR SMART ASSISTANT FOR 3D CELL CULTURE TRANSFER

The MagPen facilitates easy and fast transfer and collection of magnetised cell cultures without disrupting their microtissue architecture. Cells, magnetised and cultured by Magnetic 3D Cell Culture (M3D), can be transposed by a simple "pick up-and-drop"-step.

Additionally to that, the MagPen can be used to create and organize co-cultures by combining different magnetised 3D cell cultures.

The MagPen is availible as single version and as Multi-MagPen in 24 and 96 well format for simultaneous transfer of various cell cultures in one step.



Have a closer look to our video animation of the m3D Multi-MagPen.



#### MAGPEN -THE PRINCIPLE

- / Fast, easy and simultaneous transfer of multiple 3D cell cultures without pipetting
- Complete media change by a simple "pick up-anddrop"- step
- Simplified co-culturing of different cell types
- / Ideal for easy immunohistochemistry staining, blocking, and washing of spheroids

#### MAGNETISATION WITH NANOSHUTTLE-PL

NanoShuttle-PL is a nanoparticle assembly (~50 nm) consisting of biocompatible components: gold, iron oxide, and Poly-L-Lysine (PLL). Although NanoShuttle-PL is not itself an FDA-approved product for use in humans, the constituent components are themselves biocompatible.

The cells are magnetised by electrostatically attaching small amounts of NanoShuttle-PL non-specifically to cell membranes via PLL at a concentration of around 50 pg/cell. Magnetised cells will appear peppered with dark nanoparticles after incubation, giving a speckled appearance. A small magnetic force of 30 pN/cell is enough to levitate and assemble cells without causing any harm.

#### NANOSHUTTLE-PL - THE BIOCOMPATIBILITY

- / Will not affect proliferation, viability, metabolism, inflammatory or oxidative stress, phenotype and/ or other cell functions
- / Does not bind any specific receptors, works with all cell types
- / Will release from the cell over 7-8 days into the surrounding extracellular matrix
- Does not cause any chromosomal abnormalities in cells and does not lead to genomic instability



#### **GO DEEPER!**

Find detailed information in the related
Whitepaper at our
Download Center.



# PRODUCT OVERVIEW THINCERT® CELL CULTURE INSERTS

#### ThinCert® Cell Culture Inserts

#### 6 Well

Feature: 4 multiwell plates / box, Height: 16.25 mm, Ø internal: 24.85 mm, Ø external: 27.85 mm, Cultural surface: 452.4 mm², Surface treatment: TC, Working volume (ThinCert®): 1 ml - 3.6 ml, Working volume (well): 2 ml - 4.15 ml

Item no.	Pore density	Ø pore	Optical membrane properties	Sterile	Qty. inner / outer
657640	$1 \times 10^8  \text{cm}^2$	0.4 μm	translucent	+	1 / 24
657641	$2 \times 10^{6}  \text{cm}^{2}$	0.4 μm	clear	+	1 / 24
657610	$2 \times 10^{6}  \text{cm}^{2}$	1 µm	clear	+	1 / 24
657630	0,6 x 10 <sup>6</sup> cm <sup>2</sup>	3 µm	clear	+	1 / 24
657631	$2 \times 10^{6}  \text{cm}^{2}$	3 µm	translucent	+	1 / 24
657638	0,15 x 10 <sup>6</sup> cm <sup>2</sup>	8 µm	translucent	+	1 / 24

#### ThinCert® Cell Culture Inserts

#### 12 Well

Feature: 4 multiwell plates / box, Height: 16.25 mm, Ø internal: 13.85 mm, Ø external: 15.85 mm, Cultural surface: 113.1 mm², Surface treatment: TC, Working volume (ThinCert®): 0.3 ml - 1 ml, Working volume (well): 1 ml - 2 ml

Item no.	Pore density	Ø pore	Optical membrane properties	Sterile	Qty. inner / outer
665640	$1 \times 10^8  \text{cm}^2$	0.4 μm	translucent	+	1 / 48
665641	$2 \times 10^{6}  \text{cm}^{2}$	0.4 μm	clear	+	1 / 48
665610	$2 \times 10^{6}  \text{cm}^{2}$	1 µm	clear	+	1 / 48
665630	0,6 x 10 <sup>6</sup> cm <sup>2</sup>	3 µm	clear	+	1 / 48
665631	$2 \times 10^{6}  \text{cm}^{2}$	3 µm	translucent	+	1 / 48
665638	0,15 x 10 <sup>6</sup> cm <sup>2</sup>	8 µm	translucent	+	1 / 48

#### ThinCert® Cell Culture Inserts

#### 24 Well

Feature: 2 multiwell plates / box, Height: 16.25 mm, Ø internal: 8.4 mm, Ø external: 10.4 mm, Cultural surface: 33.6 mm<sup>2</sup>, Surface treatment: TC, Working volume (ThinCert®): 0.1 ml - 0.35 ml, Working volume (well): 0.4 ml - 1.2 ml

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Item no.	Pore density	Ø pore	Optical membrane properties	Sterile	Qty. inner / outer
662640	1 x 10 <sup>8</sup> cm <sup>2</sup>	0.4 µm	translucent	+	1 / 48
662641	$2 \times 10^{6}  \text{cm}^{2}$	0.4 μm	clear	+	1 / 48
662610	$2 \times 10^{6}  \text{cm}^{2}$	1 µm	clear	+	1 / 48
662630	$0.6 \times 10^6  \text{cm}^2$	3 µm	clear	+	1 / 48
662631	$2 \times 10^{6}  \text{cm}^{2}$	3 µm	translucent	+	1 / 48
662638	0,15 x 10 <sup>6</sup> cm <sup>2</sup>	8 µm	translucent	+	1 / 48

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## ThinCert® Plate 6 / 12 Well

Height: 39.5 mm, Length: 129.5 mm, Width: 86.6 mm, Lid: yes, condensation rings

Item no.	Well format	Working volume (well)	Sterile	Qty. inner / outer
657110	6	≤20 ml	+	1/50
665110	12	≤4 ml	+	1/60

# PRODUCT OVERVIEW CELLSTAR® CELL-REPELLENT VESSELS

#### Cell Culture Dishes

### Cell-Repellent Surface

Raw material: PS, Surface treatment: cell-repellent, Vent nock: yes

Item no.	Height	Ø nominal size	Working volume	Total volume	Sterile	Qty. inner / outer
627979	10 mm	35 mm	≤3 ml	10 ml	+	10 / 40
628979	15 mm	60 mm	6 ml - 7 ml	28 ml	+	10 / 20
664970	20 mm	100 mm	16 ml - 17 ml	100 ml	+	1/5

#### Cell Culture Flasks

#### Cell-Repellent Surface

Raw material: PS, Surface treatment: cell-repellent

Item no.	Flask design	Cap colour	Total volume	Cap design	Sterile	Qty. inner / outer
690980	flat	○white	50 ml	screw cap	+	10 / 20
690985	flat	○ white	50 ml	filter screw cap	+	10 / 20
658980	flat	○white	250 ml	screw cap	+	5 / 15
658985	flat	○ white	250 ml	filter screw cap	+	5 / 15
660980	flat	○white	550 ml	screw cap	+	5/5
660985	flat	○ white	550 ml	filter screw cap	+	5/5
661980	high	○white	650 mI	screw cap	+	4/4
661985	high	○ white	650 ml	filter screw cap	+	4/4

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#### Multiwell Plates / Microplates Cell-Repellent Surface

Raw material: PS, Surface treatment: cell-repellent

Item no.	Well format	Well profile	Bottom	Product colour	Total volume (well)	Working volume (well)	Lid	Sterile	Qty. inner/ outer
657970	6	F-bottom	solid	○ clear	16.1 ml	2 ml - 5 ml	yes, condensation rings	+	1/5
665970	12	F-bottom	solid	○ clear	6.5 ml	2 ml - 4 ml	yes, condensation rings	+	1/5
662970	24	F-bottom	solid	○ clear	3.3 ml	0.5 ml - 1.5 ml	yes, condensation rings	+	1/5
677970	48	F-bottom	solid	○ clear	1.7 ml	0.5 ml - 1 ml	yes, condensation rings	+	1/5
650970	96	U-bottom	solid	○clear	323 µl	40 μl - 280 μl	yes, condensation rings	+	1/6
650979	96	U-bottom	solid	○ clear	323 µl	40 μl - 280 μl	yes, condensation rings	+	8 / 32
651970	96	V-bottom	solid	○clear	234 μΙ	40 μl - 200 μl	yes, condensation rings	+	1/6
655970	96	F-bottom / Chimney Well	solid	○ clear	392 µl	25 µl - 340 µl	yes, condensation rings	+	1/6
655976	96	F-bottom / Chimney Well	µClear®	● black	392 µI	25 μl - 340 μl	yes, condensation rings	+	8 / 32
655976-SIN	96	F-bottom / Chimney Well	µClear®	● black	392 µI	25 μl - 340 μl	yes, condensation rings	+	1/32
781970	384	F-bottom	solid	○clear	131 μΙ	15 μl - 110 μl	yes	+	1/60
781974	384	F-bottom	μClear®	○white	131 µI	15 μl - 110 μl	yes	+	8 / 32
781976	384	F-bottom	μClear®	● black	131 µI	15 μl - 110 μl	yes	+	8 / 32
781976-SIN	384	F-bottom	μClear®	● black	131 µI	15 μl - 110 μl	yes	+	1/32
787979	384	U-bottom	solid	○ clear	122 μΙ	10 μl - 90 μl	yes	+	8 / 32

## PRODUCT OVERVIEW MAGNETIC 3D CELL CULTURE

#### Spheroid Bioprinting

### 96 Well

Item no.	Description	Content kit	Qty. inner / outer
655840	96 Well Bioprinting Kit, clear	NanoShuttle-PL (3 vials), Spheroid Drive, Holding Drive, 96 well cell culture microplates (clear) with cell-repellent surface (2 x 655970)	1/1
655841	96 Well Bioprinting Kit, black, µClear®	NanoShuttle-PL (3 vials), Spheroid Drive, Holding Drive, 96 well cell culture microplates (black, µclear®) with cell-repellent surface (2 x 655976-SIN)	1/1
655850	96 Well Ring Drive	96 Well Ring Drive for the formation of 3D ring structures	1/1
655830	96 Well Spheroid and Holding Drive	Spheroid Drive, Holding Drive	-/1

## Spheroid Bioprinting

### 384 Well

Item no.	Description	Content kit	Qty. inner / outer
781840	384 Well Bioprinting Kit, clear	NanoShuttle-PL (2 vials), Spheroid Drive, Holding Drive, 384 well cell culture microplates (clear) with cell-repellent surface (2 x 781970)	1/1
781841	384 Well Bioprinting Kit, black, μClear®	NanoShuttle-PL (2 vials), Spheroid Drive, Holding Drive, 384 well cell culture microplates (black, µClear®) with cell-repellent surface (2x 781976- SIN)	1/1
781850	384 Well Ring Drive	384 Well Ring Drive for the formation of 3D ring structures	1/1
781830	384 well spheroid and holding drive	Spheroid Drive, Holding Drive	-/1

# Magnetic Levitation 6 / 24 Well

Item no.	Description	Content kit	Sterile	Oty. inner / outer
657840	6 Well Bio-Assembler Kit	Levitation Drive, Holding Drive, NanoShuttle-PL (2 Vials), 6 well cell culture multiwell plates (2 x 657970) and 6 Well Intermediate lid (2 x 657825) with cell-repellent surface		1/1
657825	6 Well Intermediate lid	Intermediate lid with cell-repellent surface	+	2 / 10
657830	6 Well Levitation and Holding Drive	Levitation Drive (1), Holding Drive (1)		-/1
662840	24 Well Bio-Assembler Kit	Levitation Drive, Holding Drive, NanoShuttle-PL (2 Vials), 24 well cell culture multiwell plates (2 x 662970) and 24 Well Intermediate lid (2 x 662825) with cell-repellent surface		1/1
662825	24 Well Intermediate lid	Intermediate lid with cell-repellent surface	+	1 / 10
662830	24 well Levitation and Holding Drive	Levitation Drive (1), Holding Drive (1)		-/1

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#### Screening 96 / 384 Well

Item no.	Description	Content kit	Qty. inner / outer
655846	96 Well BiO Assay Kit	NanoShuttle-PL (3 vials), 6 Well Levitation Drive, 6 Well Intermediate Lid (2 x 657825) with cell-repellent surface, 96 Well Spheroid, Holding and Ring Drive, 96 Well Deep Well Plate, 6 Well cell culture multiwell plates with cell-repellent surface (2 x 657970), 96 Well cell culture microplates (clear) with cell-repellent surface (2 x 655970)	1/1
781846	384 Well BiO Assay Kit	NanoShuttle-PL (2 vials), 6 Well Levitation Drive, 6 Well Intermediate Lid (2 x 657825) with cell- repellent surface, 384 Well Spheroid and Holding Drive, 96 Well Deep Well plate, 6 Well cell culture multiwell plates with cell-repellent surface (2 x 657970), 384 Well cell culture microplates (clear) with cell-repellent surface (2 x 781970)	1/1

## MagPen

#### Single / 24 Well / 96 Well

Item no.	Description	Content kit	Sterile	Qty. inner / outer
657850	MagPen 3-pack	Teflon caps (3), magnets (3)		-/3
657824	24 Well Multi-MagPen Kit	24 Well Multi-MagPen Drive and 24 Well Multi- MagPen Sleeve (2 x 651524) with cell-repellent surface		-/1
651524	24 Well Multi-MagPen Sleeve	Multi-MagPen Sleeve with cell-repellent surface	+	1 / 10
657896	96 Well Multi-MagPen Kit	96 Well Multi-MagPen Drive und 96 Well Multi- MagPen Sleeve (2 x 61596) with cell-repellent surface		-/1
651596	96 Well Multi-MagPen Sleeve	Multi-MagPen Sleeve with cell-repellent surface	+	1 / 10

#### Consumables / Accessories

### Magnetic 3D Cell Culture

Item no.	Description	Content kit	Qty. inner / outer
657841	NanoShuttle-PL	600 μl vials of NanoShuttle-PL (1)	-/1
657843	NanoShuttle-PL 3-pack	600 µl vials of NanoShuttle-PL (3)	-/3
657846	NanoShuttle-PL 6-pack	600 μl vials of NanoShuttle-PL (6)	-/6
657852	NanoShuttle-PL 12-pack	600 µl vials of NanoShuttle-PL (12)	-/12

## making a difference



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