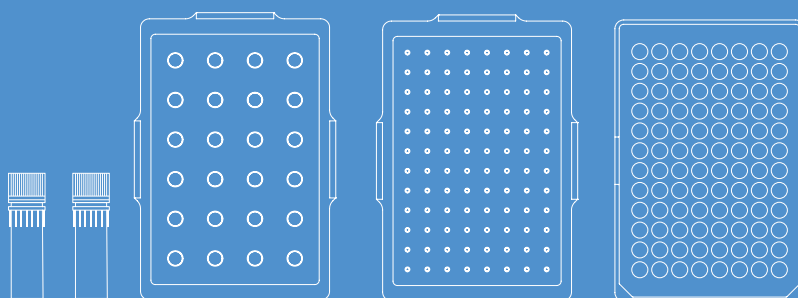


Your **Power** for Health



3D Cell Culture

3D in a fast and easy 2D workflow



3D Cell Culture in a 2D workflow

Cell culture is an essential tool in drug discovery, tissue engineering, toxicology testing, stem cell research, as well as in basic research. In 2D, cultures can only mimic the conditions of physiological tissue to a limited extent, whereas cells *in vivo* are able to interact in a three-dimensional network.

Therefore, results generated with 2D cultures may often be of limited relevance for predicting clinical effectiveness and toxicity, contributing to high attrition rates in the drug development process.¹

Cell culturing in 3D enables the expression of extracellular matrix (ECM) components as well as the formation of cell-cell and cell-matrix interactions. These characteristics are important for replicating *in-vivo* cell differentiation, proliferation, and function *in-vitro*. The employment of spheroid cultures is regarded as an improved approach to developing predictive *in-vitro* screening assays with high physiological

relevance for preclinical drug development, especially in cancer research and toxicology.

Comparisons of spheroid cultures and 2D monolayer cultures, for example of tumor cell lines, showed functional differences including alterations in protein expression, phosphorylation patterns and responsiveness to inhibitor molecules.^{2,3}

Greiner Bio-One developed CELLSTAR® cell culture vessels with cell-repellent surface to prevent cell adherence and promote spontaneous formation of three-dimensional spheroids. Round bottom plates facilitate gravitational spheroid formation, and flat bottom plates, which feature ideal optical properties for imaging and high throughput screening, are an essential accessory for the magnetic 3D cell culture technology. Both systems offer the principle and convenience of **3D in a fast and easy 2D workflow!**

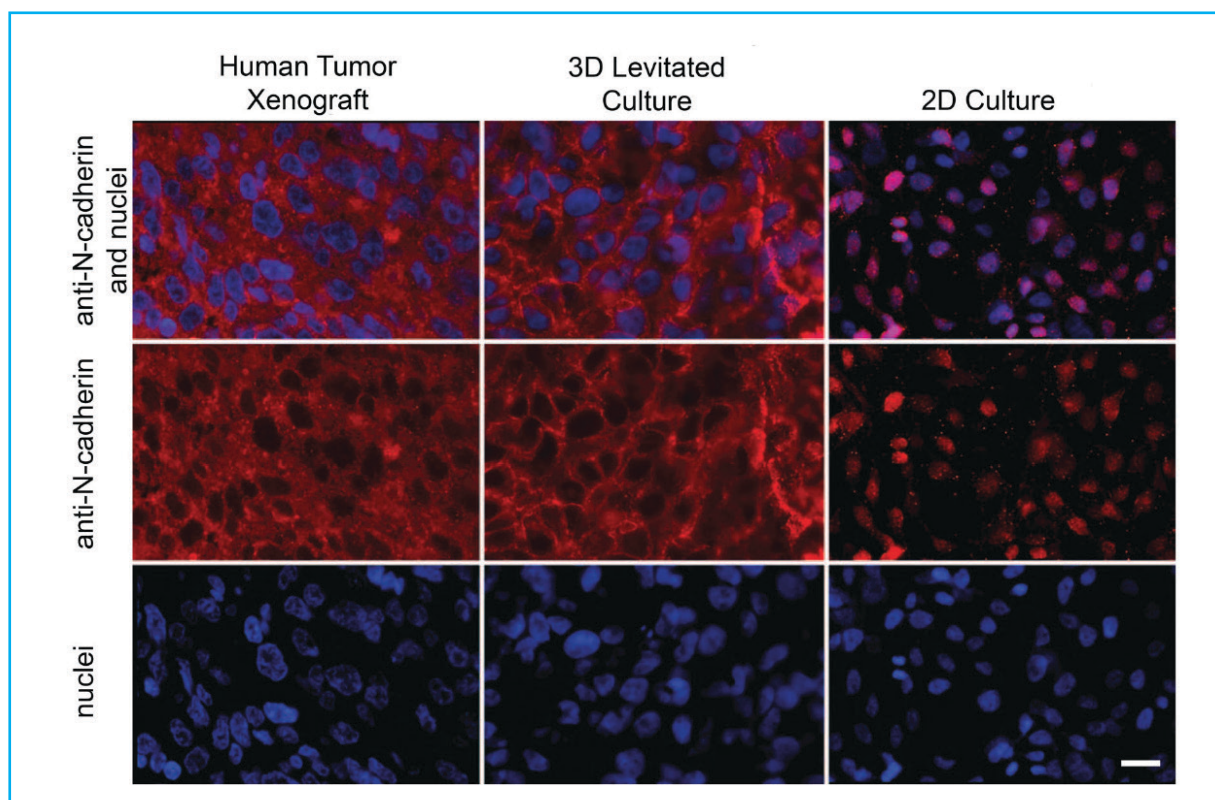


Figure 1: Immunofluorescence detection of N-cadherin (red) and DAPI nuclear staining (blue) in a mouse xenograft, 3D magnetic levitation for 48 h, and 2D standard culture with human glioblastoma cells. Scale bar, 10 μ m.

1) Friedrich J. et al. (2007). Experimental anti-tumour therapy in 3-D: Spheroids - old hat or new challenge? *Int J Rad Biol.* 83(11-12):849-871.
2) Ekert J E et al. (2014) Three-dimensional lung tumor microenvironment modulates therapeutic compound responsiveness *in vitro* – implications for drug development. *PLOS ONE* 9(3): e92248. doi:10.1371/journal.pone.0092248
3) Vinci, M et al. (2012) Advances in establishment and analysis of three-dimensional tumor spheroid-based functional assays for target validation and drug evaluation. *BMC Biology* 10:29.

CELLSTAR® Cell Culture Vessels with Cell-Repellent Surface

In contrast to standard tissue culture surfaces which are optimised to enhance conditions for cell attachment, the CELLSTAR® cell-repellent surface has been developed to effectively prevent cell attachment. For the formation of e.g. spheroids or self-assembled spherical clusters used as 3D cell culture models, the cell-cell interaction must dominate over the interaction between the cells and the culture surface of containment. Cell culture vessels equipped with the Greiner Bio-One CELLSTAR® cell-repellent surface present an ideal platform for cultivating suspension cultures of semi-adherent and adherent cell lines as well as the formation of stem cell aggregates and spheroids either with or without magnetic approach.

Features:

- ✓ **Innovative chemical surface modification**
- ✓ **Sterile**
- ✓ **4 year shelf life**
- ✓ **Free of DNase, RNase, endotoxins, no cytotoxic effects**

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 key step for cultivation and differentiation in 3D**
- ✓ **3D culture in hydrogels without cell migration out of the hydrogel and formation of 2D subcultures**
- ✓ **Indispensable part of the magnetic 3D cell culture technology**



Figure 2: Tumor cell spheroids grown in a 96 well U-bottom CELLSTAR® cell culture microplate with cell-repellent surface.

Publications and Literature

Further information on CELLSTAR® cell-repellent surface:

- ✓ Forum No. 17: CELLSTAR® Cell Culture Vessels with Cell-Repellent Surface (F073777)
- ✓ Application Report “Advantage of CELLSTAR® Cell Culture Vessels with Cell-Repellent Surface for 3D Cell Culture in Hydrogels” (F073792)
- ✓ Application Report “CELLSTAR® microplates with Cell-Repellent Surface as platform for BIOMIMESYS® (F073797)

Further information on 3D Cell Culture:

- ✓ Advanced Development of Primary Pancreatic Organoid Tumor Models for High-Throughput Phenotypic Drug Screening (F072102)
- ✓ Clinically relevant inflammatory breast cancer patient-derived xenograft-derived ex vivo model for evaluation of tumor-specific therapies (F072103)
- ✓ Engineering innervated secretory epithelial organoids by magnetic three-dimensional bioprinting for stimulating epithelial growth in salivary glands (F072130)

All publications and literature including manuals and protocols are available on www.gbo.com/3dcellculture



Magnetic 3D Cell Culture

The core technology of Greiner Bio-One's Magnetic 3D Cell Culture is the magnetisation of cells with biocompatible NanoShuttle™-PL, nanoparticles (Ø ~ 50 nm) consisting of gold and iron oxide, mixed with Poly-L-Lysine. Cell magnetisation occurs when NanoShuttle™-PL is attached electrostatically to the cell membrane during an overnight static incubation, after which cells can be aggregated by magnetic force to form structurally and biologically representative *in-vitro* 3D models, either by levitation or bioprinting. By levitating cells from 6 well or 24 well Bio-Assembler™ Kit cell-repellent plate bottoms, magnetic forces work as an invisible scaffold to gently and rapidly aggregate cells, induce cell-cell interactions and initiate ECM synthesis.

In contrast to magnetic levitation, with 3D Bioprinting in 96, 384, or 1536 well formats, 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 (15 minutes to a few hours). The magnetic system overcomes the limitations of other platforms by enabling rapid formation of spheroids, reproducible and scalable in size for high-throughput, without limitation to cell type.

When used in tandem with commercially available standardised biochemical assay methods, the 3D bioprinting method facilitates continuous assess-

ment of cell viability and other functions to provide an ideal platform for compound screening. Several methods have been derived that employ m3D technology to screen drug candidate compounds in high-throughput, high-content assays that encompass pre-animal toxicity screens, anti-cancer agents, and cardiovascular drugs, as well as many other important drug discovery targets.

Spheroids/aggregates also may be picked up and transferred between vessels using the MagPen™ magnetic tool, ideal for assembling co-cultures in both low and high throughput formats.

In addition to magnetic printing of cells with a spheroidal shape, bioprinting of cells can also be patterned into a ring formation with the 96 and 384 well BiO Assay™ Kits. For up to a 72 hour timeframe immediately following bioprinting, the patterned structures will shrink/close as a function of cell migration, viability, cell-cell interaction, and/or proliferation. This period of contraction enables a means of measurement that can be employed for rapid drug dosage screening and modeling assays, such as wound healing, aortic ring, and uterine contractility. The BiO Assay™ provides a tool to magnetically pattern 3D cell culture environments for effective prediction of an *in vivo* response *in-vitro*.

Features:

- ✓ **Reproducible**
- ✓ **Scalable – 6 Well to 1536 Well**
- ✓ **Flat well bottom surface for high-resolution microscopy and HTS**
- ✓ **Rapid 3D culture formation within hours**
- ✓ **No specialised equipment or media**
- ✓ **Easy to handle and no sample loss by utilising the magnetic forces**
- ✓ **Compatible with fluorescence microscopy, Western blotting, qRT-PCR, Flow Cytometry, viability assays, chemiluminescence, etc.**
- ✓ **Automationcapable**

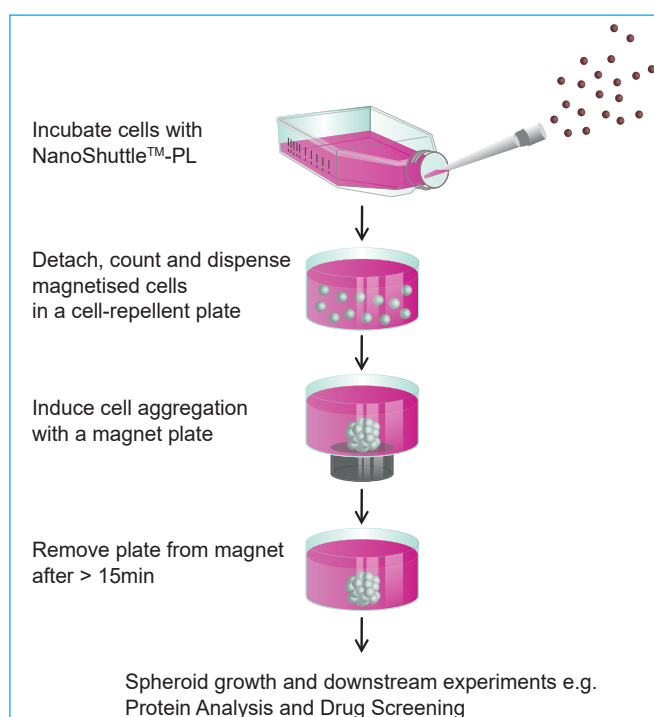


Figure 4: Basic steps of magnetic bioprinting.

Magnetic 3D Cell Culture - Applications

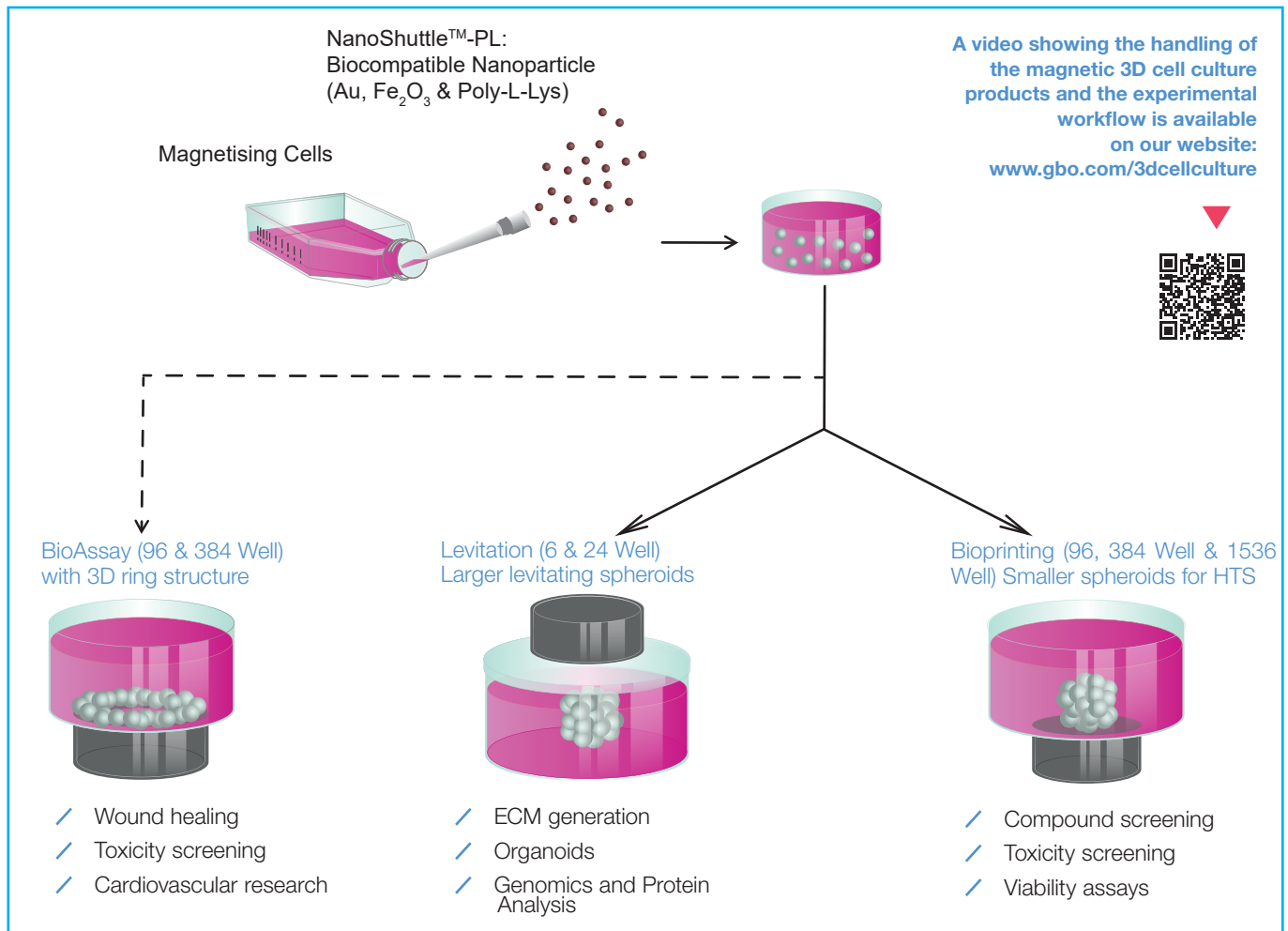


Figure 3: Magnetic 3D cell culture technology of ring formation, magnetic levitation and bioprinting for various applications.

Yes, NanoShuttle™-PL is biocompatible!

NanoShuttle™-PL is a nanoparticle assembly (~50 nm) consisting of biocompatible components: gold, iron oxide, and Poly-L-Lysine (PLL). 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. The minimal amount is enough to attract cells with mild magnetic forces of 30 pN/cell in magnetic levitation or magnetic bioprinting with fields of around 50-500 G.

Features:

- Will not affect proliferation, viability, metabolism, inflammatory or oxidative stress, phenotype and / or other macro 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
- Required magnetic forces (30 pN) do not harm cells
- Does not cause any chromosomal abnormalities in cells and does not lead to genomic instability

White paper with further information on NanoShuttle™-PL: Biocompatibility of NanoShuttle™-PL and the magnetic field in magnetic 3D bioprinting on www.gbo.com/3dcellculture



Ordering information

CELLSTAR® Cell-Repellent Surface

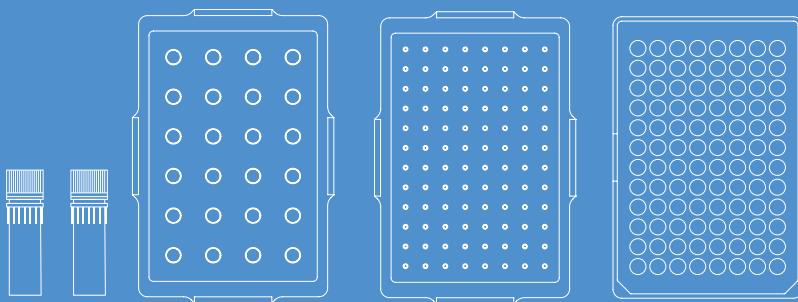
Order no.	Product Description	Qty./Bag	Qty./Case.
Cell Culture Dishes			
627979	Cell Culture Dish, Ø 35 x 10 mm, PS, cell-repellent surface, clear, sterile	10	40
628979	Cell Culture Dish, Ø 60 x 15 mm, PS, cell-repellent surface, clear, sterile	10	20
664970	Cell Culture Dish, Ø 100 x 20 mm, PS, cell-repellent surface, clear, sterile	1	5
Cell Culture Flasks			
690980	Cell Culture Flask, 50 ml, PS, cell-repellent surface, sterile, white screw cap	10	20
690985	Filter Cap Cell Culture Flask, 50 ml, PS, cell-repellent surface, sterile, white filter screw cap	10	20
658980	Cell Culture Flask, 250 ml, PS, cell-repellent surface, sterile, white screw cap	5	15
658985	Filter Cap Cell Culture Flask, 250 ml, PS, cell-repellent surface, sterile, white filter screw cap	5	15
660980	Cell Culture Flask, 550 ml, flat flask design, PS, cell-repellent surface, sterile, white screw cap	5	5
660985	Filter Cap Cell Culture Flask, 550 ml, flat flask design, PS, cell-repellent surface, sterile, white filter screw cap	5	5
661980	Cell Culture Flask, 650 ml, high flask design, PS, cell-repellent surface, sterile, white screw cap	4	4
661985	Filter Cap Cell Culture Flask, 650 ml, high flask design, PS, cell-repellent surface, sterile, white filter screw cap	4	4
Cell Culture Multiwell Plates			
657970	6 Well Multiwell Plate, PS, cell-repellent surface, clear, sterile, with lid	1	5
665970	12 Well Multiwell Plate, PS, cell-repellent surface, clear, sterile, with lid	1	5
662970	24 Well Multiwell Plate, PS, cell-repellent surface, clear, sterile, with lid	1	5
677970	48 Well Multiwell Plate, PS, cell-repellent surface, clear, sterile, with lid	1	5
Cell Culture Microplates			
655970	96 Well Microplate, PS, F-bottom/chimney well, cell-repellent surface, clear, sterile, with lid	1	6
655976	96 Well Microplate, PS, F-bottom/chimney well, cell-repellent surface, black, µClear®, sterile, with lid	8	32
655976-SIN	96 Well Microplate, PS, F-bottom/chimney well, cell-repellent surface, black, µClear®, sterile, with lid	1	32
650970	96 Well Microplate, PS, U-bottom, cell-repellent surface, clear, sterile, with lid	1	6
650979	96 Well Microplate, PS, U-bottom, cell-repellent surface, clear, sterile, with lid	8	32
651970	96 Well Microplate, PS, V-bottom, cell-repellent surface, clear, sterile, with lid	1	6
781970	384 Well Microplate, PS, cell-repellent surface, clear, sterile, with lid	1	60
781974	384 Well Microplate, PS, cell-repellent surface, white, µClear®, sterile, with lid	8	32
781976	384 Well Microplate, PS, cell-repellent surface, black, µClear®, sterile, with lid	8	32
781976-SIN	384 Well Microplate, PS, cell-repellent surface, black, µClear®, sterile, with lid	1	32
787979	384 Well Microplate, PS, U-bottom, cell-repellent surface, clear, sterile, with lid	8	32
782974	1536 Well Microplate, PS, cell-repellent, white, HiBase, µClear®, sterile, with lid	10	40
789979	1536 well Ultra Low Base SCREENSTAR Plate, CO, cell-repellent, black, flat bottom, black µClear®, sterile	17	68

Ordering information

Magnetic 3D Cell Culture

Order no.	Product description	Content / Packaging
657840	6 Well BioAssembler™ Kit	Levitating Drive, Holding Drive, 2 vials of NanoShuttle™-PL, 2x 6 Well Cell Culture Multiwell Plates with cell-repellent surface (657970) and 2x 6 Well intermediate plates (657825)
657825	6 Well intermediate plates	2 pieces per bag, 10 pieces per box
662840	24 Well BioAssembler™ Kit	Levitating Drive, Holding Drive, 2 vials of NanoShuttle™-PL, 2x 24 Well Cell Culture Multiwell Plates with cell-repellent surface (662970) and 2x 24 Well intermediate plates (657825)
662825	24 Well intermediate plates	2 pieces per bag, 10 pieces per box
655840	96 Well Bioprinting Kit clear	Spheroid Drive, Holding Drive, 3 vials of NanoShuttle™-PL and 2x 96 Well Cell Culture Microplates (clear) with cell-repellent surface (655970)
655841	96 Well Bioprinting Kit black	Spheroid Drive, Holding Drive, 3 vials of NanoShuttle™-PL and 2x 96 Well Cell Culture Microplates (black, µClear®) with cell-repellent surface (655976-SIN)
655846	96 Well BioAssay™ Kit	Spheroid Drive, Holding Drive, Levitating Drive, Holding Drive, Ring Drive, 3 vials of NanoShuttle™-PL, 96 Well Deep Well plate, 2x 96 Well Cell Culture Microplates (clear) with cell-repellent surface (655970) and 2x 6 Well Cell Culture Multiwell Plates with cell-repellent surface (657970)
655849	96 Well BioAssay™ Kit & Imaging System	Spheroid Drive, Holding Drive, Levitating Drive, Holding Drive, Ring Drive, 3 vials of NanoShuttle™-PL, 96 Well Deep Well plate, 2x 96 Well Cell Culture Microplates (clear) with cell-repellent surface (655970), 2x 6 Well Cell Culture Multiwell Plates with cell-repellent surface (657970) and Imaging Kit (657860)
781840	384 Well Bioprinting Kit clear	Spheroid Drive, Holding Drive, 2 vials of NanoShuttle™-PL and 2x 384 Well Cell Culture Microplates (clear) with cell-repellent surface (781970)
781841	384 Well Bioprinting Kit black	Spheroid Drive, Holding Drive, 2 vials of NanoShuttle™-PL and 2x 384 Well Cell Culture Microplates (black, µClear®) with cell-repellent surface (781976-SIN)
781846	384 Well BioAssay™ Kit	Spheroid Drive, Holding Drive, Levitating Drive, Holding Drive, Ring Drive, 2 vials of NanoShuttle™-PL, 96 Well Deep Well plate, 2x 384 Well Cell Culture Microplates (clear) with cell-repellent surface (781970) and 2x 6 Well Cell Culture Multiwell Plates with cell-repellent surface (657970)
781849	384 Well BioAssay™ Kit & Imaging System	Spheroid Drive, Holding Drive, Levitating Drive, Holding Drive, Ring Drive, 2 vials of NanoShuttle™-PL, 96 Well Deep Well plate, 2x 384 Well Cell Culture Microplates (clear) with cell-repellent surface (781970), 2x 6 Well Cell Culture Multiwell Plates with cell-repellent surface (657970) and Imaging Kit (657860)
781850	384 Well Ring Drive	-
789830	1536 Well Spheroid drive	-
789837	1536 Well Holding drive	-
657841	NanoShuttle™-PL Refill	-
657843	NanoShuttle™-PL Refill 3 Pack	-
657846	NanoShuttle™-PL Refill 6 Pack	-
657847	NanoShuttle™-PL Refill 6 Pack	Free iPod® with purchase of either 655849 or 781849
657852	NanoShuttle™-PL Refill 12 Pack	-
657850	MagPen™ 3 Pack	-
657860	Imaging Kit	Imaging system, light pad, cooling fan

3D Cell Culture



For further information and/or sample ordering please visit our website www.gbo.com or contact us.

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