Cell types and Publications:

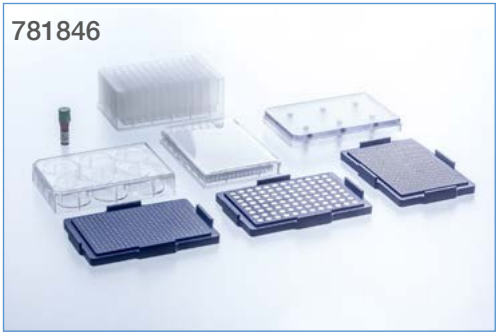
Cell types that have been successfully cultured and publications using the technology are listed on our website www.gbo.com/3dcellculture

A video showing the handling of the magnetic 3D cell culture products and the experimental workflow is available on our website www.gbo.com/3dcellculture

384-Well BioAssay Kit™ - Details and Product overview:

Order no.	Product description	Content / Packaging
781846	384-Well BioAssay Kit	384-Well Spheroid and Holding drive 6-Well Levitating drive 2 x 6-well Intermediate lid 2x Vials NanoShuttle™ 2 x 384-Well Microplates (clear) with Cell-Repellent Surface 2 x 6-Well Multiwellplate with Cell-Repellent Surface 1 x 96-Well Deep Well Plate

781846



Consumables

Order no.	Product description	Packaging
781970	384-Well Microplates, PS, F-bottom/chimney well, Cell-Repellent surface, clear, with lid sterile	1 piece / bag 60 pieces / case
657970	6-Well Multiwell Plates, PS, Cell-Repellent surface, clear, with lid sterile	1 piece / bag 5 pieces / case
657841	NanoShuttle™-PL Refill 1 Pack	1 vial / case
657843	NanoShuttle™-PL Refill 3 Pack	3 vials / case
657846	NanoShuttle™-PL Refill 6 Pack	6 vials / case
657852	NanoShuttle™-PL Refill 12 Pack	12 vials / case
657825	6-Well Intermediate lid for single use	2 pieces / bag 10 pieces / case

Troubleshooting

Problem	Probable Case	Solution
NanoShuttle™-PL appears clear	NanoShuttle™-PL has settled at the bottom of the vial	Homogenise the NanoShuttle™-PL before use by pipetting up and down at least 10 times
NanoShuttle™-PL do not appear to fully bind with cells, floating in medium	Binding with NanoShuttle™-PL varies in efficiency among cell types	NanoShuttle™-PL will appear peppered on cells and some will float, but the cells are still magnetised. Add less NanoShuttle™-PL if too excessive.
	Cells were incubated with NanoShuttle™-PL too long	Incubate cells with NanoShuttle™-PL overnight at most
Cells taking longer than usual to detach	Cells strongly adhered to substrate	Before adding trypsin, wash flask with PBS 1-2 times
NanoShuttle™-PL sparsely attached to cells	Too many cells	Increase NanoShuttle™-PL volume added to the cell culture flask to yield an ideal concentration of 1 µL/10,000 cells
Cells are sensitive to serum	Cells may undergo unwanted differentiation with serum	Use a trypsin-neutralising solution in lieu of serum-containing media to stop trypsin activity. Centrifuge cells immediately after trypsination and remove trypsin solution
Magnetised cells attaching to bottom of the plate	Magnetised cells are weakly or not bound to NanoShuttle™-PL	Use Cell-Repellent plates to prevent cells from adhering and collect weakly magnetised cells
Spheroid appears spread out	Cells have not been bioprinted for enough time	Bioprint the cells longer and carefully monitor the formation of the 3D culture
3D cultures are lost or broken when removing liquids	3D culture is not held down while liquids are transferred	Use the holding drive to hold down cultures while adding and removing liquids
When bioprinting, there are multiple spheroids per well formed	Not all cells are collected by the magnet	Use the bioprinting drive to bring cells together, and gently move the plate in circles on the desk to force magnetised cells to better aggregate
		Hold the cells longer on the magnetic drive

Warranty

The Greiner Bio-One magnet plates are warranted to be free of defects in material and workmanship for a period of 2 years from the date of purchase. The warranty is valid only if the product is used in its intended purpose and within the guidelines specified in this instruction manual. In the event that service or technical support is required, please contact your nearest Greiner Bio-One office or authorized distributor.

Revision 03: June 2020 - U072118

For further information please visit our website **www.gbo.com/3dcellculture** or contact us:

Austria	**Belgium**	**Brazil**	**China**	**France**	**Germany**	**Hungary**
office@at.gbo.com	info.be@gbo.com	office@br.gbo.com	info@cn.gbo.com	accueil.France@gbo.com	info@de.gbo.com	office@hu.gbo.com
Italy	**Japan**	**Netherlands**	**Portugal**	**Spain**	**UK**	**USA**
office@it.gbo.com	info.JP@gbo.com	info.nl@gbo.com	info@vacuette.pt	info@es.gbo.com	info.uk@gbo.com	office@us.gbo.com

Instructions for Use

Magnetic 3D Cell Culture – 384-Well BioAssay™ Kit / Order no.: 781846

The 384-Well BioAssay Kit uses NanoShuttle™-PL, a nanoparticle assembly consisting of gold, iron oxide, and poly-L-lysine to magnetise cells. By magnetic forces due to magnet plates the cells can be aggregated to form structurally and biologically representative 3D models *in-vitro*. In this kit, cells in a 384-well plate are printed into rings or spheroids using a magnetic drive to aggregate cells at the bottom of the well and culture to spheroids. The basic application of this technology is to create 3D cell cultures in a fast and easy workflow and then analyse them using common biological research techniques, such biochemical assays e.g. in drug or toxicity screenings, as well as wound-healing assays.

Intended Use

For magnetisation of cells to use in 3D cell culture. For research use only. Cell culture disposable to be used by trained personnel in a laboratory surrounding.

Safety Precautions:

To guarantee problem free and safe operation of the Bioprinting Kit please read these safety precautions before using.

- The magnet plates contain strong neodymium magnets that must be handled with extreme care.
- When storing magnets in proximity to other magnets or materials that are attracted to magnets, take precautions so that objects do not slam together. Neodymium magnets are brittle and can shatter or crack, sometimes producing dangerous fragments moving at high speeds. Fingers can also be severely pinched between magnets or between magnets and certain metals.
- Keep the magnetic drives spatially separated and DO NOT put the drives together at any time. Due to the magnetic force, placing them in close proximity can cause them
- Persons with pacemakers or similar medical devices should not come near Neodymium magnets.
- Neodymium magnets can damage magnetic media such as credit cards, magnetic ID cards, televisions, computer memory, and computer monitors. Keep magnets at least 30 cm (12 in.) from these devices away.
- Neodymium magnets should not be burned or machined. They will lose their magnetic properties if heated above 80 °C (175 °F). DO NOT AUTOCLAVE the magnetic drives.
- Neodymium magnets are not toys. The magnetic drives should only be used for their intended purpose of levitation or bioprinting cell culture. Children should not be allowed to play with them.

Store the NanoShuttle™-PL vials at 4 °C to 40 °C until first use. After fist opening of the vial, the storage recommendation is 4 °C to 8 °C. DO NOT place the NanoShuttle™-PL at temperatures below 0° C (at 1 atm) or below water freezing temperature.

Greiner Bio-One GmbH • Maybachstr. 2 • 72636 Frickenhausen • Germany
Phone: +49 (0) 7022 948-0 • Fax: +49 (0) 7022 948-514 • info@de.gbo.com • www.gbo.com/3dcellculture

Instructions for Use

1. Treating Cells with NanoShuttle™-PL

1.1. Culture cells to 80 % confluence in a T-25, T-75, or T-175 culture flask using standard procedures in your laboratory for your specific cell type.
1.2. Homogenise NanoShuttle™-PL suspension in its vial by pipetting it up and down at least 10 times.
1.3. In general add 200 µL NanoShuttle™-PL for a T-25 flask, 600 µL NanoShuttle™-PL for a T-75 flask or 1,200 µL NanoShuttle™-PL for a T-175 flask directly to the media.

! The amount of NanoShuttle™-PL added can be optimised for specific cell types by forming 3D cultures with more or less NanoShuttle™-PL before experimentation.

A benchmark concentration is 1 µL / 10,000 cells.

1.4. Incubate cells with NanoShuttle™-PL overnight. NanoShuttle™-PL is brown in color. After incubation, the cells will appear peppered with the brown NanoShuttle™-PL.

2. Cell Detachment

2.1. After incubation, warm/thaw Trypsin/EDTA solution, PBS, and media in a water bath to 37 ºC.
2.2. In a sterile hood, aspirate all media (including excess NanoShuttle™-PL) from the flask.
2.3. Wash cells to remove any remaining media and excess NanoShuttle™-PL by adding PBS to the flask and gently agitating. We recommend 2 mL of PBS for a T-25 flask, 5 mL for a T-75 flask, and 10 mL for a T-175 flask.
2.4. Aspirate PBS and add Trypsin/EDTA solution to the flask. Add enough Trypsin/EDTA solution to cover the cell monolayer, about 1 mL to a T-25 flask, 2 mL to a T-75 flask, or 4 mL to a T-175 flask. Follow your laboratory's cell-specific 2D detachment protocols.
2.5. Place the flask in an incubator for approximately 3-5 minutes or for a time prescribed by your standard protocol for detaching cells. Check for detachment under a microscope.
2.6. While waiting for cells to detach, clean the magnetic drives that you will use by wiping them with 70 % ethanol. Keep the magnetic drives sterile.

! Do not soak drives in ethanol. Lightly spray and wipe to sterilise DO NOT AUTOCLAVE the magnetic drives.

2.7. Remove flask from incubator and check under a microscope that the cells are detached from the surface. Excess exposure to Trypsin/EDTA will adversely affect cell health, so proceed to the next step quickly.
2.8. Deactivate Trypsin/EDTA by adding 37 °C media with serum. The amount of media with serum added should at least match the original volume of Trypsin/EDTA added. If cells are sensitive to serum, either use trypsin neutralising solution, or immediately centrifuge cells (at least 100 G for 5 min) and aspirate the trypsin.
2.9. Count the cells using a hemacytometer or coulter counter. Centrifuge cells and resuspend them in the required amount of media (2 mL for each well).

! We recommend levitating with 3.2×10^6 cells per well (1.6×10^6 cells/mL), but the number of cells per culture can be different. Cultures have successfully been formed with cell numbers from 5×10^6 to 1.5×10^5. Optimise the number of cells per culture by levitating cultures with more or less cells.

3. Magnetic Levitation (strongly recommended, but optional)

3.1. Dispense 2 mL of the cell suspension into each well of the 6-well plate with Cell-Repellent Surface. Do not add more than 2 mL of media as too much media in the wells will bring the cells too close to the magnet, where the cells are at risk of escaping the media.
3.2. Close the plate with the intermediate lid and place the levitating drive atop the intermediate lid (Figure 1).

! If the cells are not immediately levitating, gently agitate the plate by moving the plate back and forth, until they levitate.
When moving the plate, keep the plate flat at all times. Tilting the plate could bring the 3D culture close to the magnet, where it could escape the media.
Do not use both magnets at once, as the holding drive will tend to bring the cells to the bottom of the plate, with little to no effect from the levitating drive.

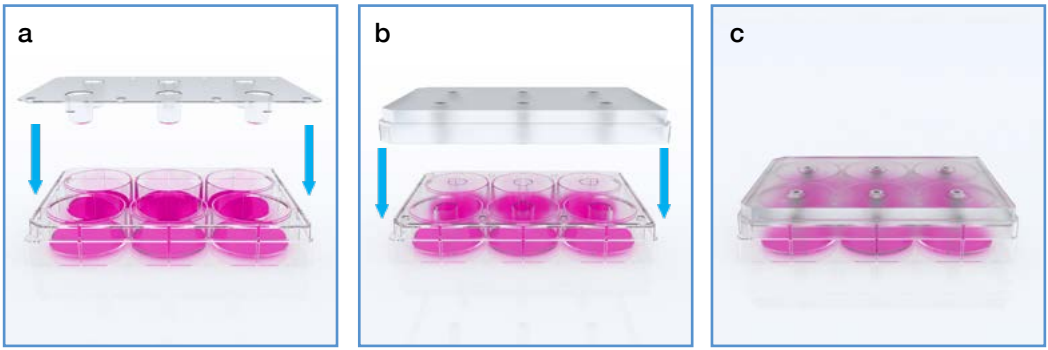


Figure 1: Place the intermediate lid atop the Cell-Repellent 6-well plate (a) and place the levitating drive atop the intermediate lid, to levitate the cells (b, c).

3.3. Transfer the plate to an incubator for up to 3h for fibroblasts and muscle cells or up to 24 h for other cell types. The purpose of this step is to induce the cells to generate extracellular matrix (ECM) and to mature, so when the cells are printed, they are in a representative environment. By 15 min, cells should begin to levitate and aggregate, forming a noticeably brown culture levitated within the well. The 3D cultures can be imaged under a microscope using the hole in the magnet where light will pass through

4. Magnetic 3D Bioprinting

4.1. Draw up the levitated structure with a sterile pipette, and break it up using vigorous pipette action, inta king and expelling the structure at least 10X. The resulting solution should be magnetised cells and ECM in suspension. Combine the levitated structures using the same cell type in a 15 mL tube.
4.2. Resuspend the cells in the required amount of media (45 µl per well).
a) For rings: 100,000 cells per ring, or a concentration of 2,222,222 cells/mL.
b) For spheroids, 25,000 cells per spheroid, or a concentration of 555,555 cells/mL.

! We recommend these cell concentrations, but the number of cells per printed structure can be diffrent. Optimise the number of cells by printing rings or spheroids with more or less cells.

For 384-Well cultures have been successfully formed with cell numbers from 120,000 to 5,000.

4.3. Place the 384-Well plate with Cell-Repellent Surface on the spheroid or ring drive, then dispense 45 µL of the cell suspension into the wells of the plate (Figure 2), respectively.

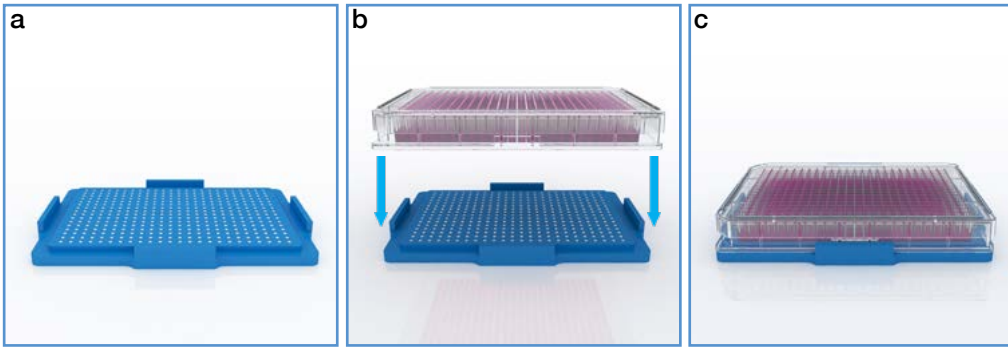


Figure 2: Take the bioprinting or ring drive (a) and place a 384-well plate with Cell-Repellent Surface atop the spheroid or ring drive (b) to aggregate the cells (c).

4.4. Carefully move the plate in circles on a flat surface to force magnetised cells to come together.
4.5. Add 5 µL of the compound solution to be tested at 10X the desired concentration to the cells. Combined, there should be 50 µL of solution (cells + compound in media) per Well.

! The compound can be added after the cells are fully printed. In adding the compound before printing, you potentially avoid disrupting the printed spheroid with the pipette. Doubly, adding the compound before printing will still yield a dose-dependent response with rapid printing times. Optimise your experiment to determine whether adding the compound before or after printing cells is best.

! While not necessary, using a multichannel pipettor to dispense the cells would reduce time exposed to the compound before printing as well as variability between wells exposed to the compound for varying amounts of time.
The amount of printing time depends on the experiment and cell type, and can vary. Typically these cells will form the spheroid by 15 min, and longer printing times allow for cell organisation, although it will plateau. Optimise the printing time by allowing the cells to print for shorter or longer.

4.6. Once printed, remove the plate off the drive. Spheroid contraction can be imaged using a microscope or real-time imager.

5. Media exchange and Post-Culture Handling

After culturing, standard tissue processing techniques can be performed on the 3D cultures, such as fixation, paraffin embedding for immunohistochemistry, or RNA isolation for qRT-PCR. Use the holding drive to hold the culture down while adding and removing liquids (Figure 2). The holding drive covers 4 wells with only magnet with the result that the spheroids are held at the rim of the well and pipetting liquids is easily possible without loss and injury of the spheroids.

5.1. Place the Cell-Repellent 384-well plate on the holding drive
5.2. Gently move the plate in circles on the desk to force magnetised cells move towards the side of the well.
5.3. Gently pipette media out placing the tip in the well at the opposite side from the magnet.

! If this is the first time exchanging media with this cell type, we suggest transferring the aspirated media to a new Cell-Repellent plate to make sure no spheroids are lost.

5.4. Before adding media, place the plate on spheroid drive to center the cultures at the center of the well during this step.
5.5. Gently move the plate in circles on the desk to force magnetised cells move to the center of the well.
5.6. Add media.
5.7. Remove the plate off the spheroid drive and visually inspect if spheroids are centered. If not, repeat the last two steps.