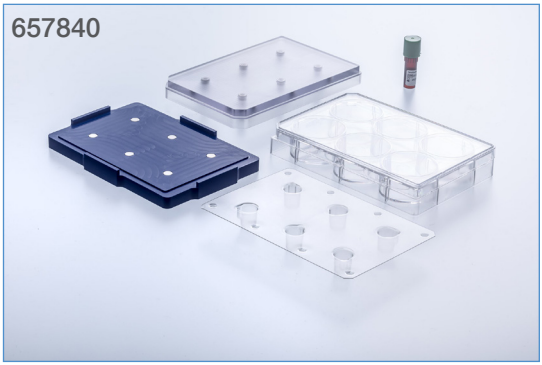
Cell types and Publications:

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

6-Well Bio-Assembler™ Kit - Details and Product overview:

Order no.	Product description	Content / Packaging
657840	6-Well Bio-Assembler™ Kit	2x Magnetic drives (Levitating and Holding drive) 2x Vials NanoShuttle™ 2x Intermediate lids 2x 6-Well Multiwell Plates with Cell-Repellent Surface



Consumables

Order no.	Product description	Packaging
657825	6-Well Intermediate Lid for single use	2 pieces / bag and 10 pieces / case
657970	6-Well Multiwell Plate, PS, Cell-Repellent Surface, clear, with lid, sterile	1 piece / bag and 5 pieces / case
657841	NanoShuttle™-PL Refill	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

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
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
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
Levitated cells are escaping the medium and attaching to the lid	Too much medium	Only add a maximum of 2 mL per well
	Plate tilted too far	Always keep the plate flat and handle it gently when moving
Levitated or concentrated cells appear spread out	Cells have not been levitated or concentrated for enough time	Place plate on holding drive for at least 5 minutes.
		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 holding drive to bring cells together, and gently move the plate in circles on the desk to force magnetised cells to better aggregate. Then, leave plate on holding drive for at least 15 minutes.
		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.

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

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Instructions for Use

Magnetic 3D Cell Culture – 6-Well Bio-Assembler™ Kit / Order no.: 657840

The 6-Well Bio-Assembler™ 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 are levitated or bioprinted in 6-well plates with a magnetic drive above or below the plate. The main application for this kit is 3D cell culture by magnetic levitation, where cells are levitated off the bottom and aggregate at the air-liquid interface to form larger 3D cultures. To magnetically bioprint cells, the holding drive is used to aggregate cells at the bottom of the well. Levitated cultures tend to be wider and spread, often allowing observation of tissue-specific morphology and organisation with lower microscope magnifications, while bioprinted cultures tend to be rounder. It is also possible to combine the two methods as desired as well. By levitating first, the cells will produce extracellular matrix, and then can be bioprinted into a more compact culture. Cells can also be bioprinted first to efficiently initiate cell-to-cell contact, then levitated to bring the spheroid to the air-liquid interface. 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 as immunohistochemical analysis, western blotting and biochemical assays e.g. drug or toxicity screenings.

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 Bio-Assembler™ 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 to “crash” together, resulting in damage to the drive magnets and / or structure.
- 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.) 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.

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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 proceduresin 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 incubtion, 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 theflask 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 tocover 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 stadard 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 thesurface. 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 serumadded 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 inthe required amount of media (2mL per culture in each well).

! We recommend levitating cultures with 1.0 x 10^6 cells per well (5 x 10^5 cells/mL), but the number of cells per culture can be different. Cultures have successfully been formed with cell numbers from 5 x 10^6 to 1.5 x 10^3. Optimise the number of cells per culture by levitating cultures with more or less cells.

3. Magnetic Levitation, Spheroid Bioprinting and Media exchange

Magnetic Levitation

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.

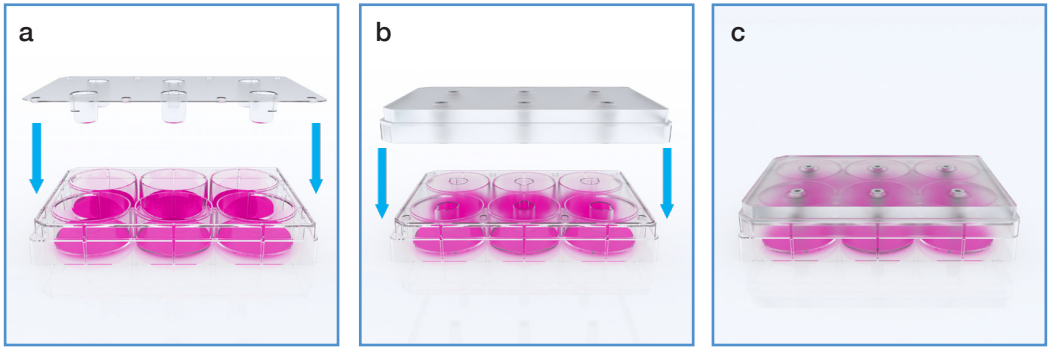

Figure 1: Place the intermediate lid atop the 6-well plate with Cell-Repellent Surface (a) and place the levitating drive atop the intermediate lid, to levitate the cells (b, c).

3.3. Transfer the plate to an incubator for the length of the experiment. By 15 min - 1 hr, cells should begin to levitate and aggregate, forming a noticeably brown culture levitated within the well. The3D cultures can be imaged under a microscope using the hole in the magnet where light can pass through. If media exchange is necessary, use the holding drive to hold the 3D cultures down while aspirating liquids (Figure 2).

3.4. Place the 6-well plate with Cell-Repellent Surface on the holding drive and dispense 2 mL of thecell suspension into the wells of the plate (Figure 2).

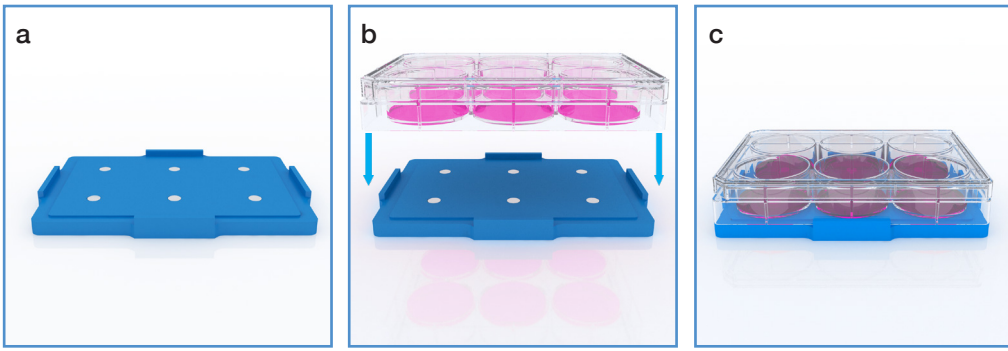

Figure 2: Take the holding drive (a) and place a 6-well plate with Cell-Repellent Surface atop the holding drive (b) to aggregate the cells or hold the 3D culture down during media exchange (c).

3.5. Gently move the plate in circles on the desk to force magnetised cells coming together.
3.6. Transfer the plate to an incubator for the length of the experiment. By 15 min - 1 hr, cells shouldbegin to aggregate, forming a noticeably brown culture at the bottom of the well. At this point, the plate can either be left on the drive or removed from the drive. The 3D cultures can only be imaged under a microscope by removing the plate from the drive.
3.7. If media exchange is necessary, use the holding drive to hold the 3D cultures down while asprating liquids (Figure 2).

Combining Bioprinting with Magnetic Levitation – most efficient approach to aggregate and levitate cells.

3.8. Follow steps 3.4 – 3.6 from Spheroid Bioprinting and Media Exchange (Figure 2)
3.9. Place the intermediate lid and place the levitating drive atop the intermediate lid (step 3.2, Figure1) to levitate the spheroid to the liquid-air interface.

4. 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).

! Protocol optimisation may be required for different cell types and / or specific experimental aims.