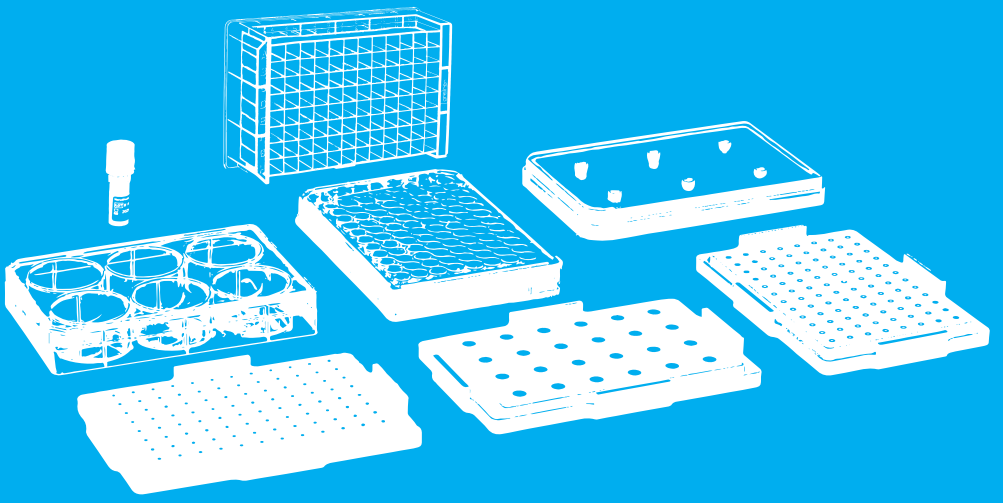
making a difference

INSTRUCTIONS FOR USE



96-WELL BIOASSAY KIT

Item No.: 655846

Generating and handling of dot and ring 3D cell culture structures by using magnetic forces

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greiner BIO-ONE

MAGNETIC 3D 96 WELL BIOASSAY KIT INSTRUCTION MANUAL

INTRODUCTION:

Thank you for purchasing this Greiner Bio-One product.

The 96-Well BioAssay Kit uses NanoShuttle-PL, a nanoparticle assembly consisting of gold, iron oxide, and poly-L-lysine to magnetise cells. In this kit, cells in a 6 and 96 well cell-repellent microplate are magnetically levitated and bioprinted to assemble/aggregate cells into 3D cultures shaped as dots or rings.

BASIC APPLICATION OF THIS TECHNOLOGY:

- **Create 3D cell cultures in a fast and easy workflow**
- **Analyze 3D cell cultures using common biological research techniques, such as biochemical assays e.g. in drug or toxicity screenings**
- **Easy handling, without the risk of sample loss**
- **Bioprinting of spheroids or 3D ring structures**
- **Wound healing assay**
- **Cell migration**

The magnets in the kit are strong, can damage electronics, and causes injury if not handled properly. Read the safety precautions carefully to ensure the correct handling of the magnets.

SAFETY PRECAUTIONS:

To guarantee problem free and safe operation of the magnetic 3D cell culture 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. Large magnets can be difficult to separate from other magnets or certain metals if they come into contact.
- DO NOT remove the magnets from the protective covers.
- 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.) 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 first 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.

1/ 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.

2/ MATERIALS:

96 Well BioAssay Kit

Item No.	Description	Content Kit	Qty. inner/outer
655846	96-Well BiOAssay Kit	NanoShuttle-PL (3 vials), 6 Well Levitation Drive, 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), 6 Well Intermediate Lid with cell-repellent surface (2 x 657825)	1 / 1

Consumables

Item No.	Description	Content / Packaging
655970	96-Well Microplates, PS, F-bottom/chimney well, Cell-Repellent surface, clear, with lid sterile	1 pieces / bag 6 pieces / case
657970	6-Well Multiwell Plates, 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

3/ OVERVIEW:

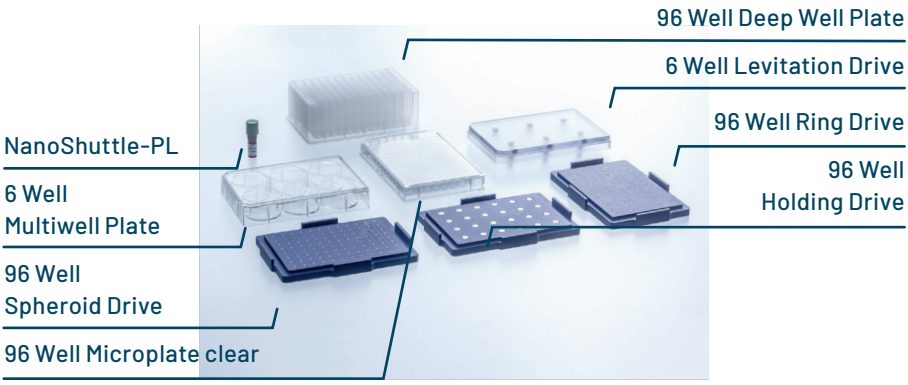


Figure 1: Item-No. 655846, 96-Well Bio Assay Kit

4/ INSTRUCTIONS:

RECOMMENDED HANDLING

INSTRUCTION TO TREAT CELLS WITH NANOSHUTTLE-PL AND HANDLING 3D CELL CULTURES

Note: Protocol optimization may be required for different cell types and/ or specific experimental aims.
Do not soak drives in ethanol. Lightly spray with ethanol and wipe to sterilise and DO NOT AUTOCLAVE the magnetic drives (see also safety precautions).

1

Treating Cells with NanoShuttle-PL
Culture cells to 80 % confluence in a culture flask using standard procedures for your specific cell type. Homogenise NanoShuttle-PL suspension by pipetting at least 10 times. Add NanoShuttle-PL directly to the media and incubate overnight.

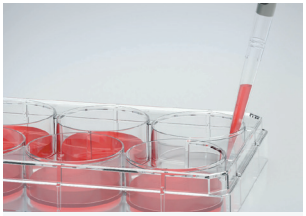
NanoShuttle-PL looks brown in color. After incubation, the cells will appear peppered with the darker/ brown NanoShuttle-PL.

i

A benchmark concentration for NanoShuttle-PL is 1 µL / 10,000 cells or in general add 200 µL for a T-25 flask, 600 µL for a T-75 flask or 1,200 µL for a T-175 flask directly to the media.
The amount of NanoShuttle-PL added can be optimized for specific cell types by forming 3D cultures with more or less NanoShuttle-PL before experimentation.

2

Wash cells to remove any remaining media and excess NanoShuttle-PL by adding PBS to the flask and gently agitating. Follow your laboratory's cell specific 2D detachment protocols.



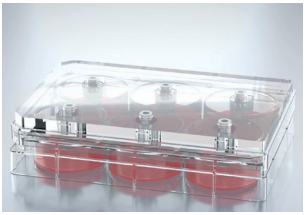
3

Magnetic Levitation
(strongly recommended, but optional)
Dispense 2 mL of the cell suspension into each well of the cell-repellent 6-well plate. 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.

i

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

4 Insert the intermediate lid as sterile barrier on the cell-repellent plate.



5 Place the levitation drive atop the intermediate lid.

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.

WARNING

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 levitation drive.

6

Transfer the plate to an incubator for up to 3 h 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 their own 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.

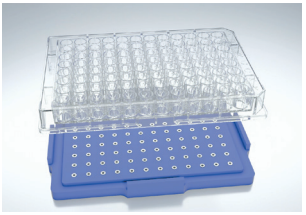
7

Draw up the levitated structure with a sterile pipette, and break it up using pipette action, intaking and expelling the structure at least 10X.

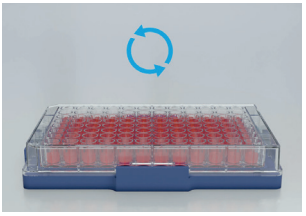
The resulting solution should be magnetised cells and ECM in suspension. Combine the levitated structures in a 15 ml tube.

8

Bioprinting for rings and spheroids
Resuspend the cells in the required amount of media (135 µL per well).
a) For rings: 200,000 cells per ring, or a concentration of approx. 1,481,481 cells/mL.
b) Suggested for spheroids: 50,000 cells per spheroid, or 370,370 cells/mL.



9 Place the 96-well cell culture plate with cell-repellent surface on the spheroid or ring drive and dispense 135 µL of the cell suspension per well.



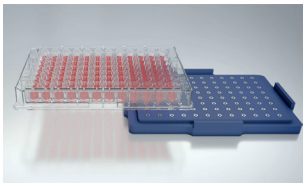
10 Carefully move the plate in circles on the desk to force magnetised cells coming together.

11 Suggested volume of 15 µL of the compound solution to be tested at 10X the desired concentration to the cells. Combined, there should be 150µL of solution (cells + compound in media) per well.

In adding the compound before printing, you potentially avoid disrupting the printed spheroid with the pipette. 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.

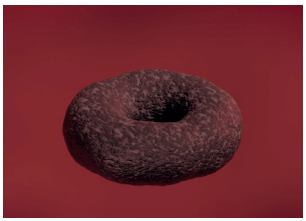
12

The amount of bioprinting time depends on the experiment and cell type, and can vary. Typically these cells will form the ring, dot or spheroid shape by 15 min, but 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.



13

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



14

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.

4/ HANDLING BEYOND THE STANDARD PROTOCOL:

1. **Media exchange**
If media exchange is necessary, use the holding drive to hold the 3D cultures down while aspirating liquids.The holding drive is designed that one magnet is adjacent to 4 wells, therefore holding the spheroids at the side of the well to pipette liquids out of the well without loss of the spheroids more easily. Place the Cell-Repellent 96-well plate on the holding drive.

2. Gently move the plate in circles on a flat surface (laminar flow hood working surface) to force magnetised cells toward the side of the well.

3. Gently pipette media out, placing the tip in the well at the opposite side from the magnet.

4. If first exchanging media with this cell type, we suggest transferring the aspirated media to a new Cell-Repellent plate to ensure no spheroids are lost in case any of the 3D cultures are held by the magnetic field.

5. Before adding media, remove the holding drive and place on the spheroid drive to keep the spheroids at the center of the well during this step.

6. Add fresh media.

7. Gently move the plate in circles on a flat surface (laminar flow hood working surface) to force magnetised cells to move to the center of the well.

8. Remove the plate off the spheroid drive and visually inspect if the spheroids are centered. If not, repeat the last two steps.

NOTES:

5/ TROUBLESHOOTING:

Problem	Problem 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 Nano-Shuttle-PL can vary in efficiency among cell types.	NanoShuttle-PL will appear pep-pered on cells and some can float, but the cells are still magnetised. Optimize amount of NanoShuttle-PL, if needed.
	Cells were incubated with NanoShuttle-PL too long.	Reduce incubation time.
Cells taking longer than usual to detach.	Cells strongly adhered to substrate.	Apply more stringent conditions to detach cells.
NanoShuttle-PL spar-sely attached to cells.	Too many cells.	Make sure cell confluence before adding Nanoshuttle-PL is approximately 80%.
Magnetised cells attaching to bottom of the plate.	The selected plate is not suitable for the application.	Use Cell-Repellent plates to prevent cells from adhering and collect weakly magnetised cells or contact the plate manufacture.
Ring/Spheroid appe-ars spread out.	Cells have not been bioprinted for enough time.	Bioprint the cells longer and care-fully monitor the formation of the 3D culture.
Ring is not fully formed.	Number of cells too low to fill the ring.	Number of cells per ring needs to increase. Carefully, but more vigorously, move the plate in circles on a flat surface to force magnetised cells to come together.
3D cultures are lost or broken when remo-ving liquids.	3D culture is not held down while liquids are removed.	Use the holding drive to hold down cultures while adding and removing liquids. After media exchange place culture plate on spheroid drive to recenter and/or reshape culture.

Problem	Problem Case	Solution
When bioprinting, there are multiple spheroids per well formed.	1. Not all cells are collected by the magnet. 2. Cells are adhering to bottom of microplate 3. Not all cells are part of the spheroids.	1. 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. 2. Use Cell-Repellent plates to prevent cells from adhering. 3. Hold the cells longer on the magnetic drive.

6/ CELL TYPES AND PUBLICATION:

Cell types that have been successfully cultured and publications using the technology are listed here.

A video showing the handling of the m3D BioAssay products and the experimental workflow is available here or by scanning the QR code.

7/ 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 case that service or technical support is required, please contact your local subsidiary or Greiner Bio-One distributor.

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