Instructions For Use

6-Well Bio-Assembler™ Kit

REF 657840
# 6-Well Bio-Assembler™ Kit Instructions For Use

Thank you for purchasing this Greiner Bio-One product. The 6-Well Bio-Assembler™ Kit uses NanoShuttle™-PL, a nanoparticle assembly consisting of gold, iron oxide, and poly-L-lysine to magnetise cells, at which point they can be magnetically directed. In this kit, cells are levitated in 6-well plates with a magnetic drive above the plate to levitate them off the bottom, where they are aggregated at the air-liquid interface to form larger 3D cultures, or printed into spheroids using a magnetic drive to aggregate cells at the bottom of the well. NanoShuttle™-PL has to be stored sterile at room temperature.

The magnets in this kit are strong, can damage electronics, and cause injury if not handled correctly. DO NOT remove the magnets from the protective covers. DO NOT autoclave. DO NOT store near metal surfaces. Read the attached instructions carefully on how to handle the magnets.

**Product Use**

The 6-Well Bio-Assembler™ Kit is for research use only. It is not approved for human or animal use.
1. MATERIALS AND SUPPLIES

<table>
<thead>
<tr>
<th>Materials and Supplies Needed to Levitate Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Well Bio-Assembler™ Kit, which includes: NanoShuttle™-PL (2x 600 µL vials); Levitating Drive (1); Concentrating/Holding Drive (1); Cell-Repellent 6-Well Plates, clear (2).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other Materials Provided by User:</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 % Ethanol</td>
</tr>
<tr>
<td>Phosphate Buffered Saline (PBS, Calcium and Magnesium free)</td>
</tr>
<tr>
<td>0.25 % Trypsin/EDTA Solution or the recommended detaching solution for your cell type</td>
</tr>
<tr>
<td>Pipettes, flasks, other general tissue culture supplies and tools</td>
</tr>
<tr>
<td>Cells (in suspension or monolayer)</td>
</tr>
<tr>
<td>Media (use typical media for 2D culture, if serum-free, use trypsin neutralisation solution to inactivate trypsin)</td>
</tr>
<tr>
<td>Microscope</td>
</tr>
<tr>
<td>Any additional supplies for the specific cell type and application</td>
</tr>
</tbody>
</table>
2. INSTRUCTIONS

Instructions for magnetically levitating cells in the 6-Well Bio-Assembler™ Kit

Overview: 600 µL of NanoShuttle™-PL will treat one T-75 flask of cells at 80 % confluence (approximately 6 million cells). At 1 x 10⁶ cells/structure, this is enough to levitate or print 6 3D cultures. 3D cultures to be paraffin-embedded may require more cells per culture. The 6-Well Bio-Assembler™ Kit works only with CELLSTAR® Cell-Repellent 6-Well Plates (REF 657970, Greiner Bio-One, included in the kit).

Optimisation may be required for different cell types or specific experimental aims.

2.1 Treating Cells with NanoShuttle™-PL

1. Culture cells to 80 % confluence in a T-25, T-75, or T-150 culture flask using standard procedures in your laboratory for your specific cell type.

2. Treat cells with NanoShuttle™-PL as follows:
   a) Homogenise NanoShuttle™-PL in its vial by pipetting it up and down at least 10 times.
   b) For a T-25 flask add 200 µL NanoShuttle™-PL, or for a T-75 flask add 600 µL NanoShuttle™-PL, or for a T-150 flask add 1,200 µL NanoShuttle™-PL directly to the media.
   c) Incubate cells with NanoShuttle™-PL overnight.

The amount of NanoShuttle™-PL added can be optimised to use more or less volume for specific cell types. Optimise the volume before experimentation by forming 3D cultures with more or less NanoShuttle™-PL added. A benchmark concentration is 1 µL/10,000 cells.

NanoShuttle™-PL is brown in color. After incubation, the cells will appear peppered with the brown nanoparticles (Figure 1).

Figure 1: After incubation with NanoShuttle™-PL, cells will appear peppered with the brown nanoparticles, as demonstrated by primary human pulmonary fibroblasts. Scale bar = 100 µm.
2.2 Cell Detachment

3. After incubation, warm/thaw Trypsin/EDTA solution, PBS, and media in a water bath to 37 °C.

4. In a sterile hood, aspirate all media (including excess NanoShuttle™-PL) from the flask.

5. 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-150 flask.

6. 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-150 flask. Follow your laboratory’s cell-specific detachment protocols.

7. 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.

8. 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.

9. 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.

10. 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.

11. Count the cells using a hemacytometer or Coulter counter. Centrifuge cells and resuspend them in the required amount of media (2 mL per culture).

   ! We recommend levitating or printing cultures with $1 \times 10^6$ cells each ($5 \times 10^5$ cells/mL), but the number of cells per culture can be different. Cultures have successfully been formed with cell numbers from $5 \times 10^5$ to $1.5 \times 10^6$. Optimise the number of cells per culture by levitating or printing cultures with more or less cells.

   ! At this point, the cells are ready for either magnetic levitation or spheroid printing. Magnetic levitation brings the cells up to the air-liquid interface, where they begin to form 3D cultures. Spheroid printing brings the cells together at the bottom of the plate to form a 3D culture. Levitated cultures tend to be more wide and spread, often allowing observation of tissue-specific morphology and organisation with lower microscope magnifications, while printed cultures tend to be more round. It is also possible to combine the two. By levitating first, the cells will produce extracellular matrix, and then can be printed into a more cohesive culture. Cells can also be printed first to form a spheroid, then levitated to bring the speroid to the air-liquid interface. The two methods can be combined as desired. However, do not use both magnets at once, as the concentrating drive will tend to bring the cells to the bottom of the plate, with little to no effect from the levitating drive.
2.3 Magnetic Levitation / Spheroid Printing

Magnetic Levitation

12a. Draw up the suspended cells with a sterile pipette, and dispense 2 mL of the cell suspension into the wells of the cell-repellent 6-well plate.

**Warning**: Too much media in the dish will bring the cells too close to the magnet, where the cells are at risk of escaping the media. Do not add more than 2 mL of media.

13a. Close the plate, place the levitating drive atop the plate (Figure 2).

**Warning**: If the cells are not immediately levitating, gently agitate the plate by moving the plate back and forth, until they levitate.

![Figure 2](image-url)

*Figure 2: Take a cell-repellent 6-well plate (a) and place the levitating drive (b) atop the cell-repellent 6-well plate to levitate the cells (c).*

14. 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. The 3D 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 3).

**Warning**: 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.
**Spheroid Printing**

12b. Place the cell-repellent 6-well plate on the concentrating drive, draw up the suspended cells with a sterile pipette, and dispense 2 mL of the cell suspension into the wells of the plate.

![Image of concentrating drive and 6-well plate](image)

**Figure 3**: Take the concentrating drive (a) and place a cell-repellent 6-well plate (b) atop the concentrating drive to aggregate the cells (c).

14. Transfer the plate to an incubator for the length of the experiment. By 15 min - 1 hr, cells should begin 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. If media exchange is necessary, use the holding drive to hold the 3D cultures down while aspirating liquids (Figure 3).

![Warning]

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.

### 2.4 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 3).
### 3. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Probable Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>NanoShuttle™-PL appears separated</td>
<td>NanoShuttle™-PL has settled at the bottom of the vial</td>
<td>Homogenise the NanoShuttle™-PL before use by pipetting up and down 10X</td>
</tr>
<tr>
<td>NanoShuttle™-PL do not appear to fully bind with cells, floating in medium</td>
<td>Binding with NanoShuttle™-PL varies in efficiency among cell types</td>
<td>NanoShuttle™-PL will appear peppered on cells and some will float, but the cells are still magnetised. Add less NanoShuttle™-PL if too excessive</td>
</tr>
<tr>
<td></td>
<td>Cells were incubated with NanoShuttle™-PL too long</td>
<td>Incubate cells with NanoShuttle™-PL overnight at most</td>
</tr>
<tr>
<td>Cells taking longer than usual to detach</td>
<td>Cells strongly adhered to substrate</td>
<td>Before adding trypsin, wash flask with PBS 1-2X</td>
</tr>
<tr>
<td>NanoShuttle™-PL sparsely attached to cells</td>
<td>Too many cells</td>
<td>Increase NanoShuttle™-PL volume added to each well to yield an ideal concentration of 1 µL/10,000 cells</td>
</tr>
<tr>
<td>Cells are sensitive to serum</td>
<td>Cells may undergo unwanted differentiation with serum</td>
<td>Use a trypsin-neutralising solution in lieu of serum-containing media to stop trypsin activity. Centrifuge cells immediately after and remove trypsin solution</td>
</tr>
<tr>
<td>Magnetised cells attaching to bottom of the plate</td>
<td>Magnetized cells are weakly or not bound to NanoShuttle™-PL</td>
<td>Use cell-repellent plates to prevent cells from adhering and collect weakly magnetised cells</td>
</tr>
<tr>
<td>Levitated cells are escaping the medium and attaching to the lid</td>
<td>Too much medium</td>
<td>Only add a maximum of 2 mL per well</td>
</tr>
<tr>
<td></td>
<td>Plate tilted too far</td>
<td>Always keep the plate flat when moving</td>
</tr>
<tr>
<td>Levitated or concentrated cells appear spread out</td>
<td>Cells have not been levitated or concentrated for enough time</td>
<td>Levitate or concentrate the cells longer and carefully monitor the formation of the 3D culture</td>
</tr>
<tr>
<td>3D cultures are lost or broken when removing liquids</td>
<td>3D culture is not held down while liquids are transferred</td>
<td>Use the holding drive to hold down cultures while adding and removing liquids</td>
</tr>
</tbody>
</table>
4. CELL TYPES

Cell types that have been successfully cultured using the procedure include:

Cell lines
- Murine Endothelial Cells
- Murine Embryonic Fibroblasts, pre-adipocytes (3T3)
- Murine Adipocytes
- Murine Melanoma
- Murine Neural Stem Cells
- Rat Hepatoma
- Human Astrocytes
- Human Glioblastoma Multiforme (LN229)
- Human Embryonic Kidney Cells (HEK293)
- Rat Vascular Smooth Muscle Cells (A10)
- Human Hepatocellular Carcinoma (HepG2)
- Human Lung Adenocarcinoma (A549)
- Human Colorectal Carcinoma (HCT116)
- Human Pancreatic Epithelioid Carcinoma (PANC-1)

Primary cells
- Human Pulmonary Microvascular Endothelial Cells
- Human Tracheal Smooth Muscle Cells
- Human Small Airway Epithelial Cells
- Human Pulmonary Fibroblasts
- Human Mesenchymal Stem Cells
- Human Bone Marrow Endothelial Cells
- Human Umbilical Vein Endothelial Cells
- Human Aortic Vascular Smooth Muscle Cells
- Human Neonatal Dermal Fibroblasts
- Murine Chondrocytes

5. REFERENCES

Germany
Greiner Bio-One GmbH
P: +49 7022 948-0
F: +49 7022 948-514
E: info@de.gbo.com

Austria
Greiner Bio-One GmbH
P: +43 7583 6791-0
F: +43 7583 6318
E: office@at.gbo.com

Belgium
Greiner Bio-One BVBA/ SPRL
P: +32 2461 0910
F: +32 2461 0905
E: info@be.gbo.com

Brazil
Greiner Bio-One Brasil
P: +55 19 3468-9600
F: +55 19 3468-9621
E: office@br.gbo.com

China
Greiner Bio-One Suns Co., Ltd.
P: +86 10 83 55 19 91
F: +86 10 63 56 69 00
E: office@cn.gbo.com

France
Greiner Bio-One SAS
P: +33 1 69 86 25 25
F: +33 1 69 86 25 35
E: accueil@gbo.com

Hungary
Greiner Bio-One Hungary Kft.
P: +36 96 213 088
F: +36 96 213 198
E: office@hu.gbo.com

Italy
Greiner Bio-One Italia S.r.l
P: +39 02 94383340
F: +39 02 94383366
E: office@it.gbo.com

Japan
Greiner Bio-One Co. Ltd.
P: +81 3 3505 8875
F: +81 3 505 8974
E: info@jp.gbo.com

Netherlands
Greiner Bio-One B.V.
P: +31 17 2420 900
F: +31 17 2443 801
E: info@nl.gbo.com

Portugal
Vacuette Portugal S.A.
T: +351 252 647721
F: +351 252 647722
E: info@vacuette.pt

Spain
Vacuette Espana S.A.
T: +34 91 6527707
F: +34 91 6523335
E: info@vacuette.es

United Kingdom
Greiner Bio-One Ltd.
P: +44 1453 8252 55
F: +44 1453 8262 66
E: info@uk.gbo.com

USA
Greiner Bio-One North America Inc.
P: +1 704 261-7800
F: +1 704 261-7899
E: office@us.gbo.com