Instructions For Use

Single-Well Bio-Assembler™ Kit

REF 627840
Thank you for purchasing this Greiner Bio-One product. The Single-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 35-mm petri dishes with a magnetic drive above the dish to levitate them off the bottom, where they are aggregated at the air-liquid interface to form larger 3D cultures. 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 Single-Well Bio-Assembler™ Kit is for research use only. It is not approved for human or animal use.
1. MATERIALS AND SUPPLIES

Materials and Supplies Needed to Levitate Cells

Single-Well Bio-Assembler™ Kit, which includes: NanoShuttle™-PL (2x 600 µL vials); Single-Well Levitating Drives (3); Single-Well Concentrating/Holding Drives (3); Cell-Repellent 35-mm Petri Dishes (10); Holding Lid (1).

Other Materials Provided by User:

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

Instructions for magnetically levitating cells in the Single-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 6 3D cultures. 3D cultures to be paraffin-embedded may require more cells per culture. The Single-Well Bio-Assembler™ Kit works only with CELLSTAR® Cell-Repellent 35-mm Petri Dishes (REF 627979, Greiner Bio-One, included in the kit).

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 1200 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 NanoShuttle™-PL (Figure 1).

![Figure 1](Illustration.png)

*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 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 cultures with more or less cells.
### 2.3 Magnetic Levitation

12. Draw up the suspended cells with a sterile pipette, and dispense 2 mL of the cell suspension into the cell-repellent 35-mm petri dish.

**Tip:**
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.

13. Close the dish and place a single-well magnetic drive atop the dish (Figure 2). Place the dishes in the holding lid to hold.

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

**Tip:**
Two magnet sizes come with this kit, concentrating/holding (small) or levitating (large) (Figure 2). While the levitating magnet has a stronger magnetic field, the concentrating magnet has a smaller magnetic field with which to bring cells closer together. Magnet size has no effect on cell behavior, only on the morphology of the 3D culture.

*Figure 2: Magnetised cells dispensed into a cell-repellent 35-mm petri dish are levitated by placing either the levitating (large) or concentrating (small) drive atop the dish.*

14. Transfer the dishes to an incubator for the length of the experiment. By 15 min - 1 h, 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. If media exchange is necessary, use the single-well holding drive to hold the 3D cultures down while aspirating liquids by placing it below the dish with the magnet facing upwards (Figure 3).

**Tip:**
When moving the dishes, keep them flat at all times. Tilting the dishes 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, such as fixation, paraffin embedding for immunohistochemistry, or RNA isolation for qRT-PCR. Use the single-well holding drive to hold the culture down while adding and removing liquids by placing it below the dish with the magnet facing upward (Figure 3).

Figure 3: Use the holding drive to hold 3D cultures as you add and remove liquids.
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

## 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>Cells were incubated with NanoShuttle™-PL too long</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>Magnetised 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>Levitated cells appear spread out</td>
<td>Plate tilted too far</td>
<td>Always keep the plate flat when moving</td>
</tr>
<tr>
<td>Levitated cells appear spread out</td>
<td>Cells have not been levitated for enough time</td>
<td>Levitate 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>
For further information and/or sample ordering please visit our website www.gbo.com/3dcellculture or contact us:

**Germany (Main office)**
Greiner Bio-One GmbH
E-Mail info@de.gbo.com

**Austria**
Greiner Bio-One GmbH
E-Mail office@at.gbo.com

**Belgium**
Greiner Bio-One BVBA/SPRL
E-Mail info.be@gbo.com

**Brazil**
Greiner Bio-One Brasil
E-Mail office@br.gbo.com

**China**
Greiner Bio-One Suns Co., Ltd.
E-Mail info@cn.gbo.com

**France**
Greiner Bio-One SAS
E-Mail accueil.France@gbo.com

**Hungary**
Greiner Bio-One Hungary Kft.
E-Mail office@hu.gbo.com

**Italy**
Greiner Bio-One Italia S.r.l
E-Mail office@it.gbo.com

**Japan**
Greiner Bio-One Co. Ltd.
E-Mail info.JP@gbo.com

**Netherlands**
Greiner Bio-One B.V.
E-Mail info.nl@gbo.com

**Portugal**
Vacuette Portugal S.A.
E-Mail info@vacuette.pt

**Spain**
Greiner Bio-One España
E-Mail info@es.gbo.com

**UK**
Greiner Bio-One Ltd.
E-Mail info.uk@gbo.com

**USA**
Greiner Bio-One North America Inc.
E-Mail office@us.gbo.com