Contact information

Nano3D Biosciences, Inc.
University Tower Center
7000 Fannin Street
Suite 2140
Houston, TX 77030

Phone: 713-790-1833
info@n3dbio.com

Distributed in Austria, Germany and Switzerland by PELOBIOTECH GmbH

PELOBIOTECH GmbH
Am Klopferspitz 19
82152 Planegg / Germany

Contact us:
(P) +49 (0)89 517 28 59-0
(F) +49 (0)89 517 286 59-88
Email:info@pelobiotech.com
www.pelobiotech.com

Instruction Sheet

Instruction Sheet for the
Bio-Assembler™
Single-Well Configuration with
Nanoshuttle™-PL

Instruction Sheet for the Bio-Assembler™ Single-Well Configuration with Nanoshuttle™-PL

Thank you for purchasing this Nano3D, Biosciences product. The **Bio-Assembler™** uses magnetic levitation to enable fast and simple three-dimensional cell culturing without artificial scaffolds or matrices. **Nanoshuttle™-PL** is an animal-origin-free, polylysine-based nano-assembly that delivers magnetic nanoparticles to cells so that they can be manipulated with magnetic fields to manipulate cells. **Nanoshuttle™-PL** should be stored at 4°C.

Caution

Magnets are strong and can damage electronics and cause injury if not handled correctly. **DO NOT** remove magnets from the protective covers. Read the attached magnet-handling instructions carefully.

Product Use

The **Bio-Assembler™** is for research use only. It is not approved for human or animal use.

Materials and Supplies Needed for Single-Well Experiments
Nanoshuttle™-PL (200 µL per T-25 tissue culture flask, 600 µL per T-75 tissue culture flask)
Single-well Bio-Assembler™ magnetic drives (small or large magnets in protective covers)
Single-Well Petri Dishes, 35 mm x 10 mm (see below for recommendations)
70% Ethanol
PBS Buffer (Calcium and Magnesium free)
0.25% Trypsin/EDTA Solution or the recommended detaching solution for your cell type
Pipettes
1 x 95 mm x 15 mm petri dish holder
Large Flasks, T-75 (75 cm^2) or Small Flasks, T-25 (25 cm^2)
Cells
Cell Media*
Inverted Microscope
Additional supplies may be needed for specific applications

*Use the media type that is typically used to culture your cells of interest in 2D. (If it does not contain serum, you will also need serum to inactivate the Trypsin/EDTA.)

Figure 1. Seven single-well petri dishes with large-magnet Bio-Assembler™ magnetic drives on top.

Instruction for culturing cells with the Bio-Assembler™ single-well configuration starting with surface-attached cells:

Overview: 600 µl of **Nanoshuttle™-PL** will treat three T-25 flasks of cells at 80% confluence (approximately 3 million cells). This is typically enough cells for seeding twenty **35 mm x 10 mm petri dishes**. Experiments requiring large tissue samples, such as paraffin tissue embedding, may require twice as many cells per well. The Bio-Assembler™ was designed to work with dishes containing 2.5 ml of media. This yields a 7.5 mm distance from the magnet bottom to the bottom of the media meniscus where the levitating cells reside.

Two different magnetic drives are available for use with single-well petri dishes. The single-well magnetic drive with small magnet will concentrate the cells under the magnet aperture, which is ideal for *in situ* imaging. The magnetic drive with large magnet will result in higher yield of levitated cells, but the levitated tissue will reside to the side of the aperture. The use of petri dishes with a depth different from 10 mm or a different amount of media may affect the efficiency of the Bio-Assembler™ system. Standard adhering dishes will result in approximately 70% levitation yield with the large magnetic drive, and non-treated or non-adhering petri dishes will improve the yield. Optimization may be required for different cell types or specific experimental aims.

1. Culture cells to 80% confluence in T-25 or T-75 cell culture flasks using standard procedures in your laboratory for your specific cell type. Treat cells with **Nanoshuttle™-PL** as follows:

a) Remove **Nanoshuttle™-PL** from refrigeration and let it stand at room temperature for at least 15 minutes.

b) Homogenize **Nanoshuttle™-PL** in its vial by pipetting it up and down at least 10 times.

c) Add NanoshuttleTM-PL directly to the media in the following quantities:

- For T-25 (25 cm^2) tissue culture flask, add 200 µl of **Nanoshuttle™-PL**.
- For T-75 (75 cm^2) tissue culture flask, add 600 µl of **Nanoshuttle™-PL**.

d) Gently agitate flask to evenly distribute **Nanoshuttle™-PL**. **Note: Nanoshuttle™-PL** has a darkened coloration. The cells will display this coloration after being treated with **Nanoshuttle™-PL**.

2. Incubate treated cells in their flask for at least 5 hours using your laboratory's standard cell culture incubation conditions. Overnight incubation is acceptable.

3. After the incubation period is complete, warm/thaw Trypsin/EDTA, PBS buffer, and Media in a water bath to about 37°C.

4. Remove and discard all media (including excess of **Nanoshuttle™-PL**) from the culture flask with a sterile pipette.

5. Wash cells to remove any remaining media and excess **Nanoshuttle™-PL** by adding PBS to the flask and gently agitating. We recommend:

- For a T-25 tissue culture flask, add 5 mL of PBS.
- For a T-75 tissue culture flask, add 10 mL of PBS.

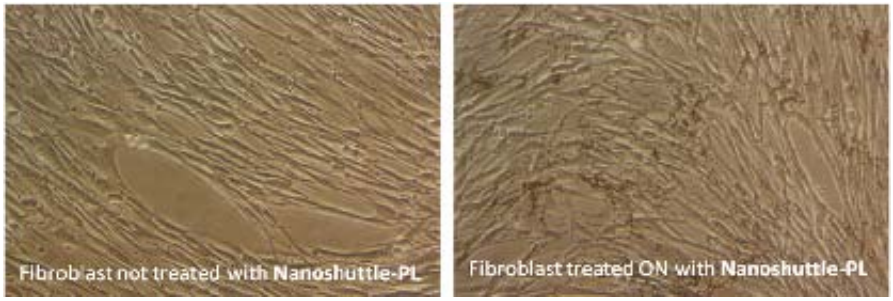

Figure 2. Human fibroblast cells plated in a T-25 flask before treatment (left) and after treatment with Nanoshuttle™-PL, incubation overnight, and application of PBS (right). Magnification is 10x.

6. Remove PBS with a pipette and discard it. To begin detaching cells, add enough Trypsin/EDTA solution to cover the adhering cell layer. Detachment protocols are cell specific. If your laboratory has an established protocol, we suggest you follow it. If not, we suggest:

- For T-25 tissue culture flask, add 1 mL of Trypsin/EDTA solution.
- For T-75 tissue culture flask, add 2 mL of Trypsin/EDTA solution.

Contact n3D for additional help. Optimization may be required.

7. Place the vessel in an incubator for approximately 3 minutes or for a time prescribed by your standard protocol for detaching cells.

8. While waiting for cells to detach, clean the magnetic drives that you will use by wiping them with 70 % ethanol.

9. Remove flask from incubator and check culture with an inverted microscope to be sure that the cells are adequately 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 serum or media containing serum. The volume added must be at least as large as the volume of Trypsin/EDTA used. We suggest;

- For T-25 tissue culture flask, add 1 mL of serum/media.
- For T-75 tissue culture flask, add 2 mL of serum/media.

11. Draw the cell suspension with a sterile pipette. Expel the suspension back into the flask to rinse the inside surfaces. Repeat several times. Check again under a microscope to ensure that cells are in suspension. Redraw the entire cell suspension into the pipette. Cells should then be counted using a hemacytometer or Coulter counter.

12. If needed, dilute solution with media to a desired density to facilitate adding a specific number of cells to each culture vessel. For optimum results with levitating cultures, seeding of at least 150,000 cells per 35 mm x 10 mm petri dish is recommended.

13. Place the bottoms of the desired number of 35 mm x 10 mm petri dishes inside the large 95 mm x 15 mm petri dish holder.

14. Load and levitate cells as follows:
    a) Ensure there is no settling of cells in the pipette by expelling the suspension into a suitable container and redrawing. Repeat several times.
    b) Add desired amount of cell suspension and additional media if needed to each 35 mm X 10 mm petri dish. Total fluid in dish should be 2.5 ml.
    c) Place a cover on each petri dish.
    d) Place one magnetic drive on top of each 35 x 10 mm petri dish cover. The exposed side of the magnet should be oriented down. A large or small magnet may be used, depending upon your specific experiment. (See the overview for more details.)

Note: The time between adding cell suspension to dishes and placing the magnetic drives should be kept short and consistent for optimal and reproducible levitation results.

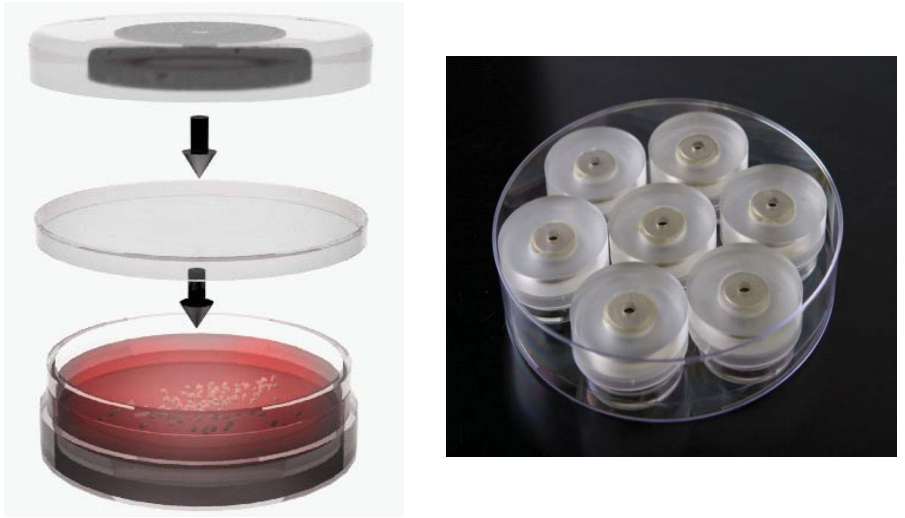

Figure 4. (Left) Assembly of petri dish and magnetic drive. (Right) Petri dishes in petri dish holder. The small-magnet magnetic drive is shown.

15. Gently agitate the petri dish holder for 10 seconds. Ensure that media does not come close to magnets. Failure to do so may lead to the deposition of cells on the underside of the petri dish cover.

16. Gently place holder with petri dishes in an incubator and incubate. Take care while moving holder to keep dishes level to ensure that media does not come close to magnets.

17. Cells may be removed from the incubator for diagnostics or the addition of new reagents. Care should be taken to minimize the agitation of the dishes and to keep them level as described above. Agitation may disturb the formation of large-scale tissue structure, especially during the first day of culturing. The magnetic drive may be removed, and the cells will typically drop to the bottom of the dish. They will re-levitate when the magnetic drive is reapplied, but this may disturb the formation of large-scale tissue structure. After several days of culturing, most cell types form large-scale tissue structure that is robust against agitation and removal of the magnetic drive.

18. Cells can be visualized using a standard inverted optical microscope. Position petri dishes in the 95 mm x 15 mm petri dish holder on the microscope stage and pass light though the opening in the center of the magnetic drive on the dish of interest. Using 4x, 10x, or 20x magnification, it should be possible to visualize levitating 3D cell cultures. If higher magnification is needed, remove an individual dish from holder and remove its magnetic drive. Wait approximately 1 minute for cells to drop to the bottom of petri dish for improved imaging. After imaging, replace the magnet on top of the cover of the petri dish. The cells should readily re-levitate.

19. If necessary, feed 3D cultures after 3 days by adding or replacing media. New media should be fresh and 37°C before adding it to the petri dish.

Caution: If replacing media, carefully remove the old media with a pipette so the 3D structures are not lost. The safest way to change media is to place the magnet under the bottom of the petri dish before doing any pipetting. The exposed side of the magnet should now be oriented up. The cells will be magnetically held on the bottom of the dish while you change the media.

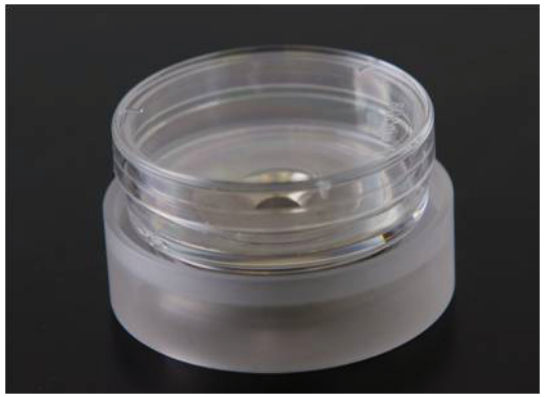

Figure 5. Magnet placed at the bottom of petri-dish.

Note: If desired, after 48 hours of levitation, levitating 3D cell structures can be transferred to a new 35 mm x 10 mm petri dish containing fresh media. To do so, carefully pipette the 3D structures from each dish with a 1 ml or 2 ml pipette into the new dish. Repeat for each dish.

Post-culture handling of 3D structures:
After cells are cultured, remove magnetic drive and wait for cells to drop to the bottom of the petri dish. Standard laboratory procedures for cell fixation, paraffin embedding or freezing, sectioning, and staining may then be used.

Cell Types

All cell types that have been tested with the **Bio-Assembler™** using the procedure described below have been cultured successfully. This includes:

Cell lines

- Murine Endothelial
- 3T3 Fibroblasts, pre-adipocytes
- Murine Adipocyte
- Murine Melanoma
- Murine Neural Stem Cells
- Rattus Norvegicus Hepatoma
- Human Astrocytes
- Human Glioblastoma Multiforme (GBM) LN 229
- Human Embryonic Kidney 293 (HEK 293)

Primary cells

- Human Pulmonary Microvascular Endothelial Cells (HPMEC)
- Human Tracheal Smooth Muscle Cells (HTSMC)
- Human Small Airway Epithelial Cells (HSAEpiC)
- Human Pulmonary Fibroblasts (HPF)
- Human Mesenchymal Stem Cells (HMSC)
- Human Bone Marrow Endothelial Cells (HBMEC)
- Human Umbilical Vein Endothelial Cells (HUVEC)
- Murine Chondrocytes

Nano3D Biosciences believes that the technology is broadly applicable to essentially all cell types. If you would like to discuss other cell types with our technical support, please call: *713-790-1833***.**

Notes: