

Instructions For Use

384-Well Bioprinting Kit (clear plates)

REF 781840

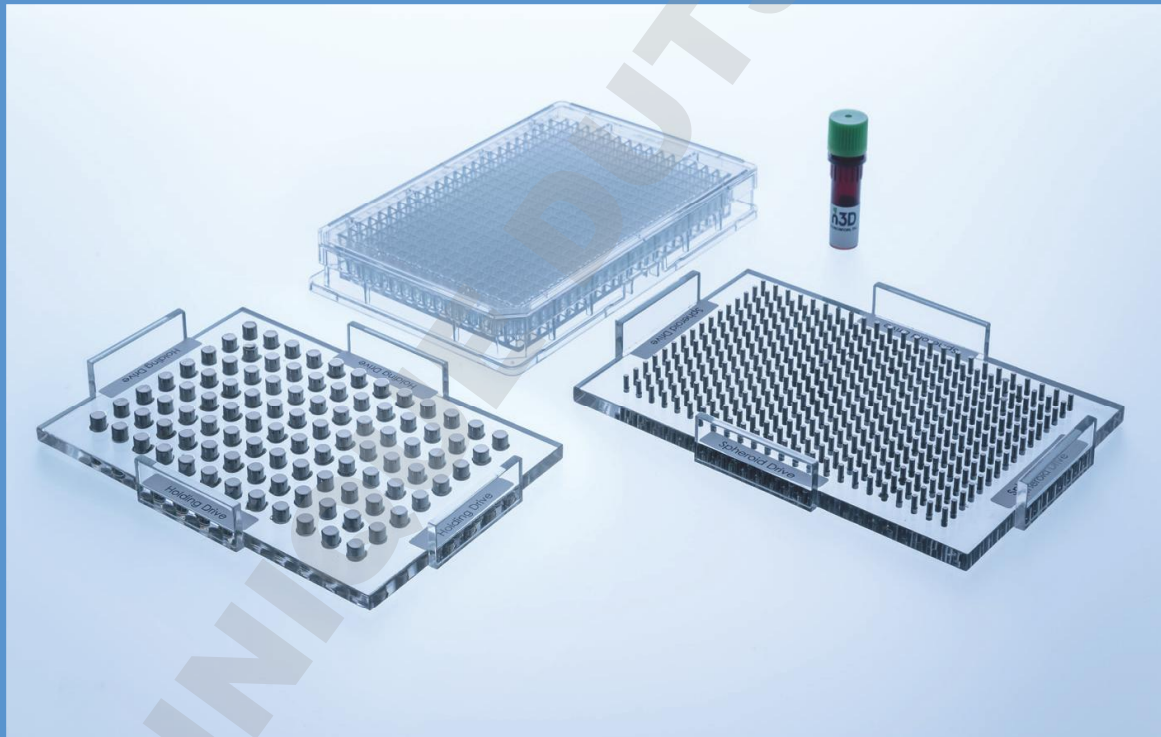


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384-Well Bioprinting Kit Instruction For Use

Thank you for purchasing this Greiner Bio-One product. The 384-Well Bioprinting 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 in a 384-well plate are 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 384-Well Bioprinting Kit is for research use only. It is not approved for human or animal use.

1. MATERIALS AND SUPPLIES

Materials and Supplies Needed to Make Spheroids
384-Well Bioprinting Kit, which includes: NanoShuttle™-PL (2x 600 µL vials); Spheroid Drive (1); Holding Drive (1); Cell-Repellent 384-Well Plates, clear (2).
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)
Medium (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 forming spheroids in the 384-Well Bioprinting Kit

Overview: 600 μL of NanoShuttle™-PL will treat one T-75 flask of cells at 80 % confluence (approximately 6 million cells). At 5,000 cells/spheroid, this is enough to form 1,200 spheroids. Spheroids to be paraffin-embedded may require more cells per spheroid. The 384-well Bioprinting Kit works only with CELLSTAR® Cell-Repellent 384-Well Plates (REF 781970, 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 $\mu\text{L}/10,000$ cells.



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

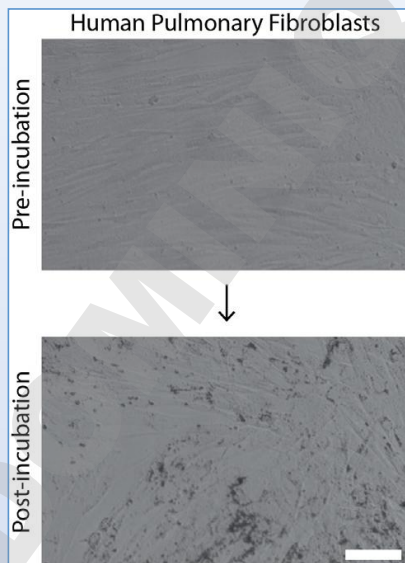


Figure 1: After incubation with NanoShuttle™-PL, cells will appear peppered with the brown nanoparticles, as demonstrated by primary human pulmonary fibroblasts. Scale bar = 100 μm^1

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 themagnetic 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 (50 µL per spheroid).



We recommend forming spheroids with 5,000 cells each (100,000 cells/mL), but the number of cells per spheroid can be different. Cultures have successfully been formed with cell numbers from 50,000 to 50. Optimise the number of cells per spheroid by forming spheroids with less cells.

2.3 Spheroid Printing

- Place a cell-repellent 384-well plate atop the spheroid drive (Figure 2).
- Dispense the cells into the plate with 50 μL of solution per well and close the plate. The cells within the solution will aggregate at the bottom of the well plate in the shape of the magnet. Leave the plate on the spheroid drive in the incubator for 15 min to a few hours to yield a competent spheroid.



Longer printing times, although possible, may not be necessary, as the magnet will aggregate cells very quickly. Optimise the printing time for your specific experiment so that the resulting spheroid can be removed from the magnet and still maintain its structure.

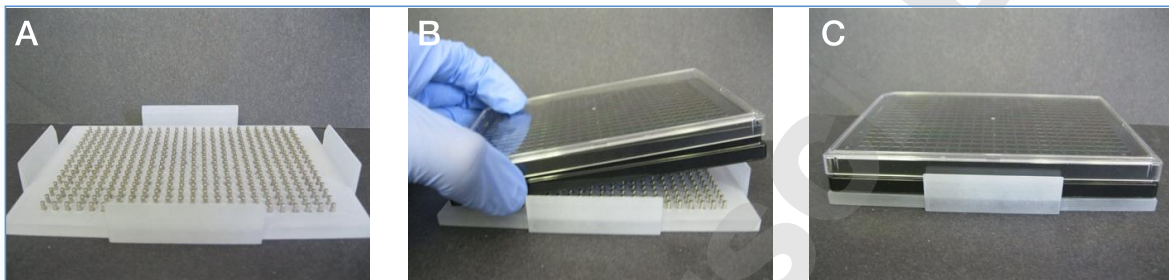


Figure 2: Take the spheroid drive (a) and place a cell-repellent 384-well plate (b) atop the spheroid drive to print the cells into a spheroid (c).

- After printing, remove the plate off the drive and transfer it back to the incubator for the length of the experiment. The spheroids can be cultured up to 3 weeks. If necessary, replace the media in the wells after 2-3 days of culture. Use the holding drive to hold the spheroids down while aspirating solutions to prevent unwanted cell loss (Figure 3).

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 holding drive to hold cells down while adding and removing liquids (Figure 3).

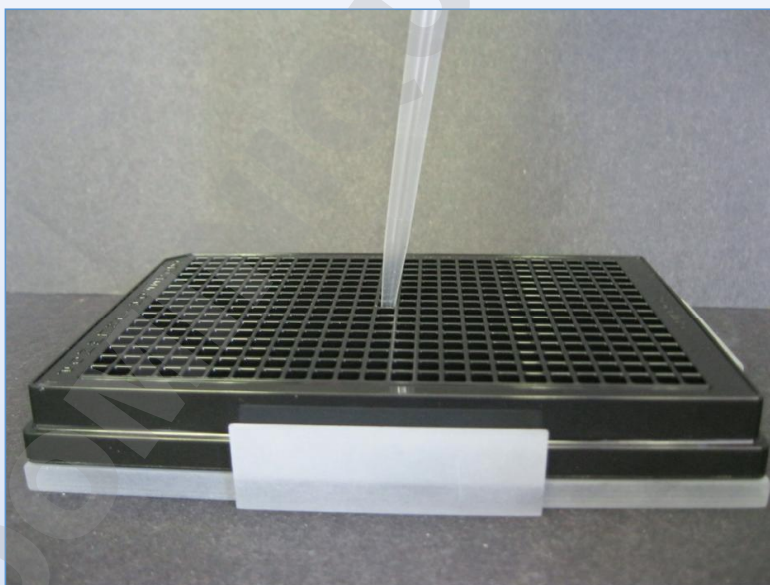


Figure 3: Use the holding drive to hold 3D cultures as you add and remove liquids

3. TROUBLESHOOTING

Problem	Probable Cause	Solution
NanoShuttle™-PL appears separated	NanoShuttle™-PL has settled at the bottom of the vial	Homogenise the Nano Shuttle™-PL before use by pipetting up and down 10X
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
Cells taking longer than usual to detach	Cells strongly adhered to substrate	Before adding trypsin, wash flask with PBS 1-2X
NanoShuttle™-PL sparsely attached to cells	Too many cells	Increase NanoShuttle™-PL volume added to each well 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. Centrifuge cells immediately after and remove trypsin solution
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
Spheroid appears spread out	Cells have not been bioprinted for enough time	Print the cells longer and carefully monitor the formation of the spheroid
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

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

¹ Haisler, W. L. et al. Three-dimensional cell culturing by magnetic levitation. Nat. Protoc. 8, 1940–9 (2013).

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

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