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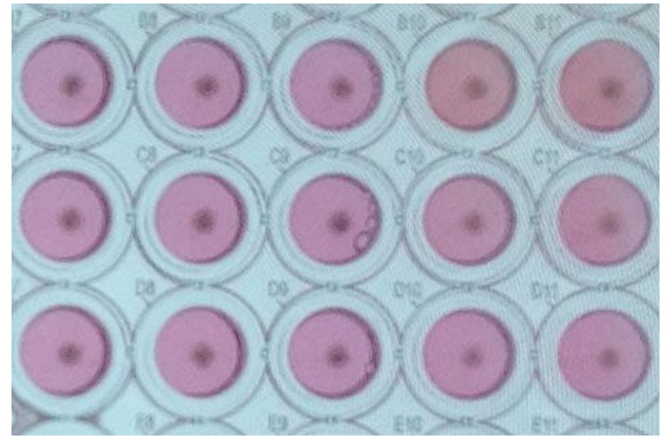
Instruction Manual
96-Well Bioprinting Kit
(black plates)

(Cat. No. 655 841)
Revision: 11/2015



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96-Well Bioprinting Kit Instruction Manual

Thank you for purchasing our product for 3D cell culturing. The **96-Well Bioprinting Kit** uses **NanoShuttle™-PL,** a nanoparticle assembly consisting of gold, iron oxide, and poly-L-lysine to magnetize cells, at which point they can be magnetically directed. In this kit, cells in a 96-well plate are printed into spheroids using a magnetic drive to aggregate cells at the bottom of the well. **NanoShuttle™-PL** should be stored at 4°C.

Caution

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 **96-Well Bioprinting Kit** is for research use only. It is not approved for human or animal use.

Notes

Notes

Materials and Supplies

Materials and Supplies Needed to Make Spheroids
96-Well Bioprinting Kit, which includes: NanoShuttle™-PL (3 600 μL vials); 96-Well Spheroid Drive (1); 96-Well Holding Drive (1); Cell-Repellent 96-Well Plates, black, μClear® (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 neutralization solution to inactivate trypsin)
Microscope
Any additional supplies for the specific cell type and application

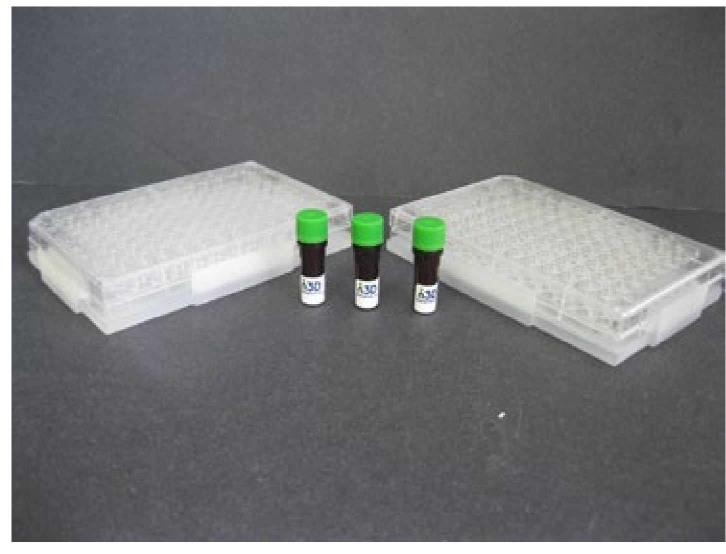


Fig. 1: 96-Well Bioprinting Kit

Instructions for forming spheroids in the 96-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 50,000 cells/spheroid, this is enough to form 120 spheroids. Spheroids to be paraffin-embedded may require more cells per spheroid. The **96-well Bioprinting Kit** works best with CELLSTAR® Cell-Repellent 96-Well Plates, black, µClear® (Cat. No. 655 976, Greiner Bio-One, included in the kit).

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

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) 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) 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.

d) Incubate cells with **NanoShuttle™-PL** overnight.

Note: The amount of **NanoShuttle™-PL** added can be optimized to use more or less volume for specific cell types. Optimize the volume before experimentation by forming spheroids with more or less **Nanoshuttle™-PL** added. A benchmark concentration is 1 µL/10,000 cells.

Note: NanoShuttle™-PL is brown in color. After incubation, the cells will appear peppered with the brown **NanoShuttle™-PL** (Fig. 2).

Some of the cell types that have been successfully cultured using the procedure include:

Cell lines

- Murine Endothelial
- Murine Embryonic Fibroblasts, pre-adipocytes (3T3)
- Murine Adipocyte
- Murine Melanoma Murine Neural Stem Cells
- Rat Hepatoma
- Human Astrocytes
- Human Glioblastoma Multiforme (GBM) LN 229
- Human Embryonic Kidney (HEK293)
- Rat Vascular Smooth Muscle (A10)
- Human Hepatocellular Carcinoma Cells (HepG2)
- Human Lung Adenocarcinoma Cells (A549)
- Human Colorectal Carcinoma Cells (HCT116)
- Human Pancreatic Epithelioid Carcinoma (PANC-1)

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)
- Human Aortic Vascular Smooth Muscle (HASMC)
- Human Neonatal Dermal Fibroblasts (HDFn)
- Murine Chondrocytes

References

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

Problem	Probable Cause	Solution
NanoShuttle™-PL appears separated	**NanoShuttle™-PL** has settled at the bottom of the vial	Homogenize the **NanoShuttle™-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 magnetized. 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-neutralizing solution in lieu of serum-contained media to stop trypsin activity. Centrifuge cells immediately after and remove trypsin solution
Magnetized cells attaching to bottom of the plate	Magnetized cells are weakly or not bound to **NanoShuttle™-PL**	Use cell-repellent plates to prevent cells from adhering and collect weakly magnetized cells
Spheroid appears spread out	Cells have not been printed 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 96-well holding drive to hold down spheroids while adding and removing liquids

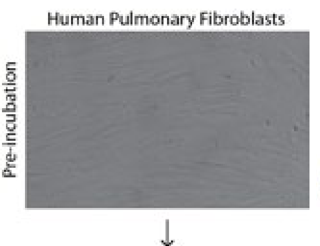
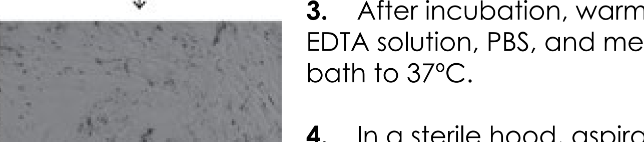


Fig. 2: After incubation with NanoShuttle™-PL, cells will appear peppered with the brown nanoparticles, as demonstrated by primary human pulmonary fibroblasts. Scale bar = 100 µm.[1]

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.

Note: Do not soak drives in ethanol. Lightly spray and wipe to sterilize.

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 neutralizing 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 (150 µL per spheroid).

Note: We recommend forming spheroids with 50,000 cells each (333,333 cells/mL), but the number of cells per spheroid can be different. Cultures have successfully been formed with cell numbers from 100,000 to 40,000. Optimize the number of cells per spheroid by forming spheroids with less cells.

Spheroid Printing

12. Place a cell-repellent 96-well plate atop the 96-well spheroid drive (Fig. 3).

13. Dispense the cells into the plate with 150 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 drive for 15 min to a few hours to yield a competent spheroid.

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

Fig. 3: Take a 96-well spheroid drive (a) and place a cell-repellent 96-well plate (b) atop the 96-well spheroid drive to print the cells into a spheroid (c).

Instructions

15. After printing, remove the plate off the drive and transfer it to an 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 96-well holding drive to hold the spheroids down while aspirating solutions to prevent unwanted cell loss (Fig. 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 96-well holding drive to hold cells down while adding and removing liquids (Fig. 4).

Fig. 4: Use the 96-well holding drive to hold 3D cultures as you add and remove liquids