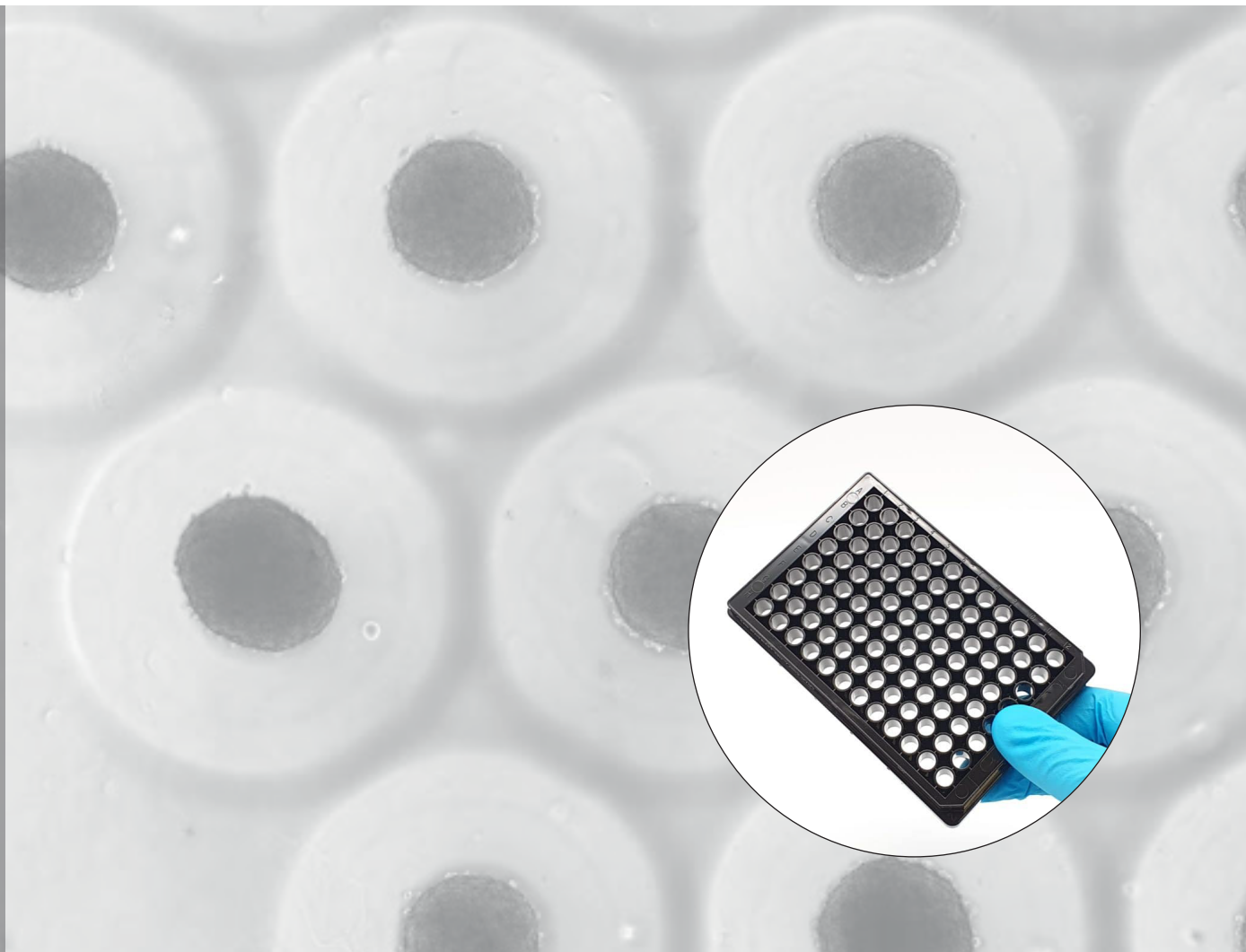
4Dcell
EXPLORE BETTER.

4Dcell SmartSphero Plates
96 well format - 19 microwells
User protocol

USER PROTOCOL



4Dcell SmartSphero Plates User Guide

How to use 4Dcell anchored spheroid plates: 96 wells

Materials included

Glass-bottom plates with polyethylene glycol-based microstructured hydrogels facilitating spheroid formation.

Materials not included

10 - 1000 µL micropipette
Distilled water
PBS

Shipping & storage

The plates are shipped in sealed sterile bags. The gels inside of the wells are submerged in an aqueous solution encapsulated with a protective film. The plates can be stored at room temperature for up to three months from the date of production.

Before starting

To avoid contamination, only manipulate the plates using aseptic techniques in a laminar flow hood.

The hydrogel structures are bound to the surface of the glass bottom in each well. To preserve the integrity of the structures, avoid touching the gels with any hard or sharp objects and always keep them submerged.

This assay was optimized for mesenchymal stem cells, with every spheroid containing approximately 5000 cells, meaning 95000 cells per gel. The protocol may need adjustments to satisfy your specific needs. The number of cells per seeding well can be changed according to the desired spheroid size or for your specific cell type by adjusting the seeding concentration.

Please refer to the schematic below and the appendix of this document for definitions and nomenclature.

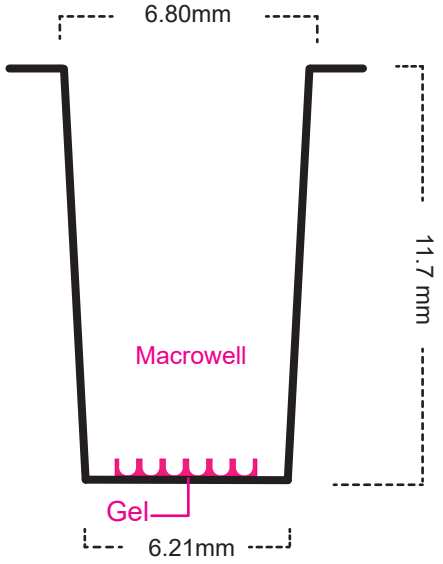


Fig. 1: Schematic cross-section of a well (not to scale).

Protocol

a. Using an aseptic technique in a laminar flow hood, open the sealed bag and expose the well by gently removing the sealing film.

b. Aspirate the storage liquid, making sure not to touch the gel structure located in the middle of the well. 150 µL

c. Rinse each gel by adding 200 µL of PBS to each well.

d. Remove the PBS, taking care not to touch the gel to avoid damaging it[(1)].

e. Add 100 µL of culture medium to each well and incubate for 15 minutes under cell culture conditions.

f. While the gels are incubating, prepare the cell solution to the desired concentration[(2)]. A total volume of 15ml of cell suspension is necessary to seed the entire 96 well plate.

g. Move the plate back into the laminar flow hood and remove the medium. Gently pipet 150 µL of cell suspension straight on top of the gel, without touching it.

h. Once every well is seeded, transfer the plate into an incubator and incubate in cell culture conditions.

i. We recommend waiting at least 8 hours before changing the medium to allow the cells to sediment and form spheroids[(3)]. To change the medium, remove the medium from the well and add 150 µL of pre-warmed medium. To ensure that the medium is sufficiently replenished, the medium can be changed twice.

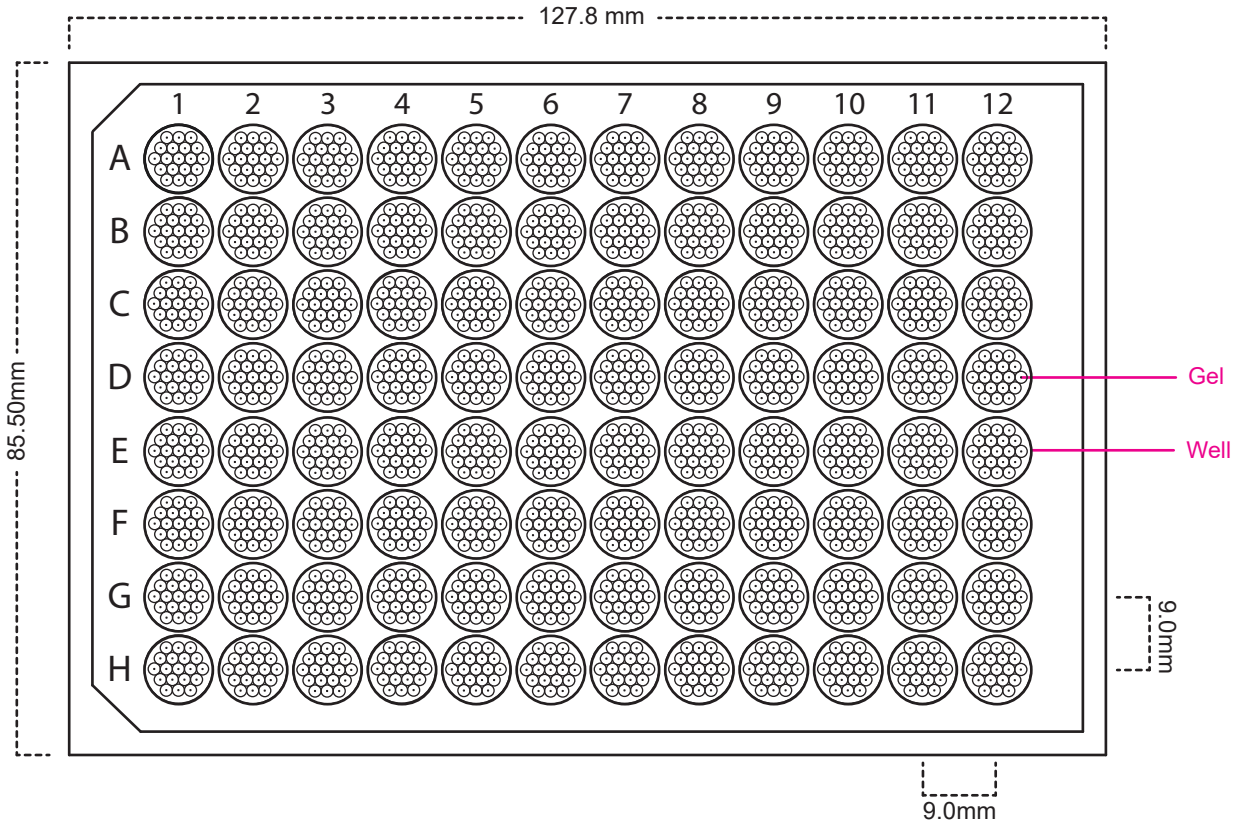
***j**. Optional: In order to harvest the spheroids from the microwells for further analysis, pipette medium vigorously straight over the gel using a 1 mL pipette until the spheroids detach. Alternatively, spheroids can be detached enzymatically by adding 200 µL of trypsin to each well and incubating for 2-3 minutes.*

Notes

(1) To avoid going over the gel and distorting it, the plate can be tilted slightly.
(2) The concentration, and thus the number of cells per spheroid, can be modified according to the cell type of the desired spheroid size. We recommend a seeding density of 5000 cells/spheroid (95 000 cells/well for 19 microwells, or 6.3 x 10^5 cells/mL)
(3) This period of sedimentation and spheroid formation may vary according to the cell type.

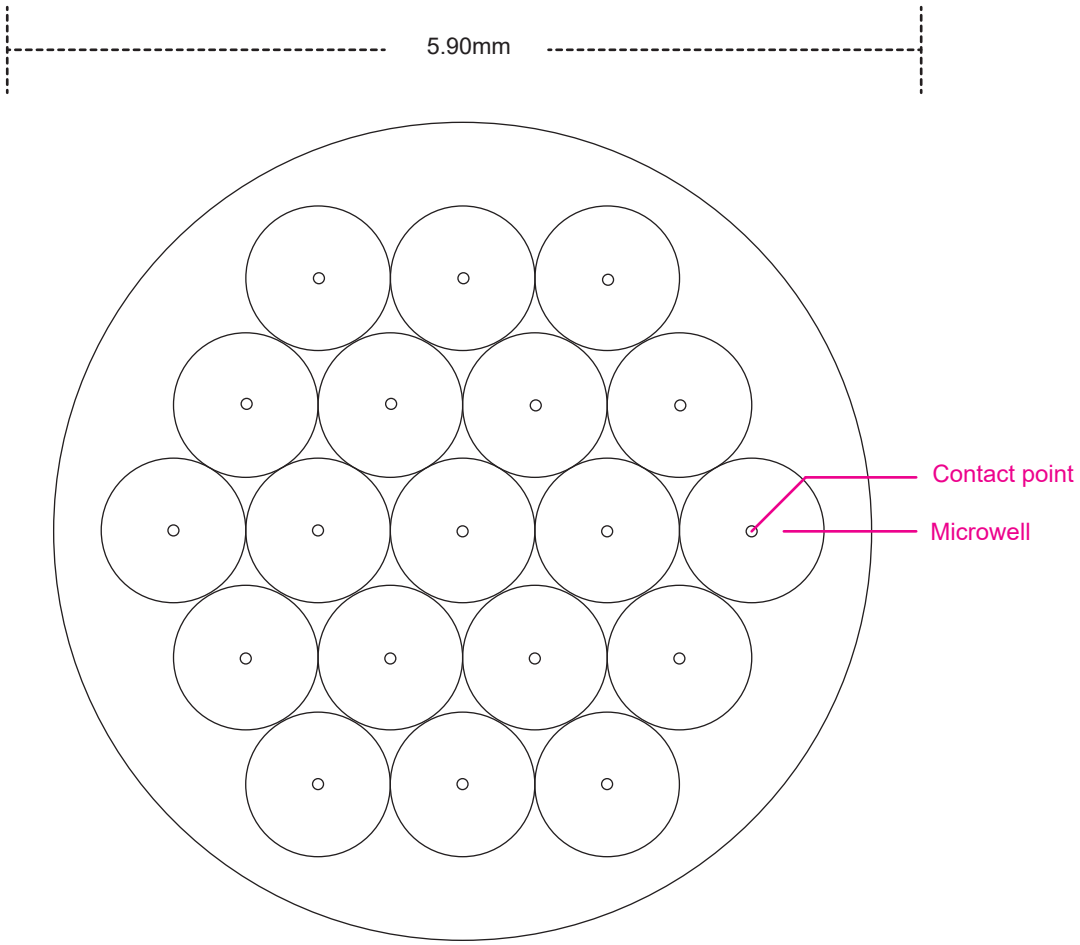
Appendix

96-well plate

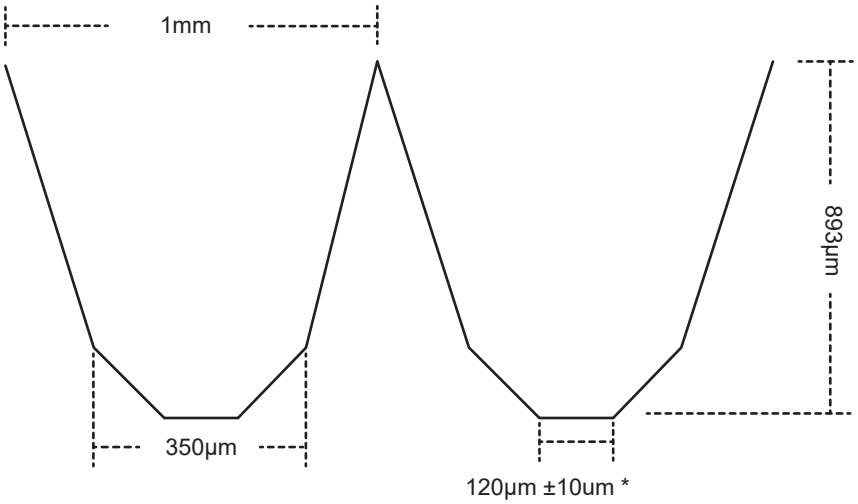


Appendix

Microstructured gel



Cross-section microwell



* Customizable