

**4Dcell Anchored Spheroid  
24-well plate**  
User protocol

USER PROTOCOL



# 4Dcell spheroids User Guide

## How to use 4Dcell (anchored) spheroid plates: 24 wells

### Materials included

Glass-bottom plates with microstructured gels facilitating spheroid formation.

### Materials not included

10 - 1000  $\mu$ L micropipette  
Distilled water  
PBS

### Optional

#### > Coating solution

*e.g. fibronectin, collagen, laminin. The fibronectin solution provided by 4Dcell has a concentration of 50  $\mu$ g/ml. It can be adjusted according to the cell type.*

### 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 one month 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 a glass coverslip in the middle of each seeding 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 3000 cells, meaning 273.000 cells per gel. The protocol may need adjustments to satisfy your specific needs. The number of cells per gel 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.

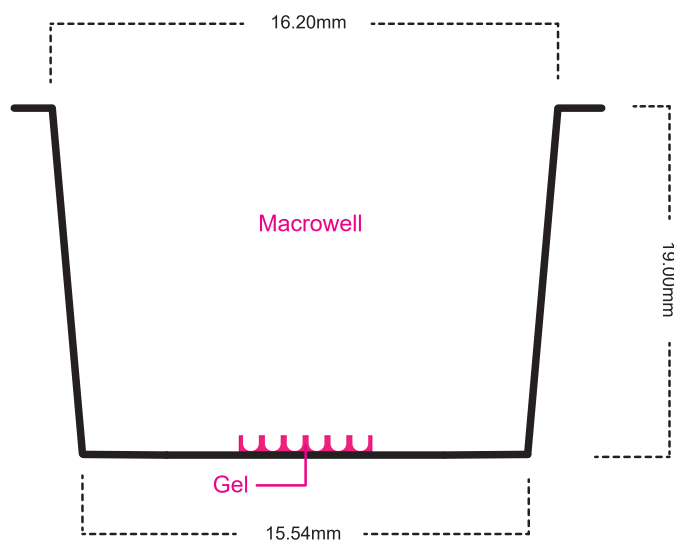


Fig. 1: Schematic cross-section of a well (not to scale).

## 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.

**If coating with an adhesion protein is necessary, proceed to step b.1. Otherwise, proceed directly to step c.**

**b.1.** *Optional: Pipette 100  $\mu$ L of your adhesive protein onto each gel<sup>(1)</sup>.*

**b.2.** *Optional: Incubate for 30 minutes to 1 hour at room temperature.*

**b.3.** *Optional: Discard the adhesive protein solution from the wells.*

**b.4.** *Optional: Rinse each gel with 2 mL of distilled water in the macro well before proceeding to the cell seeding step.*

**c.** Rinse each gel by adding 2mL of PBS to the well.

**d.** Remove the PBS, taking care not to touch the gel to avoid damaging it<sup>(2)</sup>.

**e.** Add 500  $\mu$ L of culture medium to the macro well and incubate for 15 minutes under cell culture conditions.

**f.** While the gels are incubating, prepare the cell solution to the desired concentration<sup>(3)</sup>. A total volume of 12ml of cell suspension is necessary to seed the entire 24-well plate.

**g.** Move the plate back into the laminar flow hood and remove the medium. Gently pipet 500  $\mu$ L of cell suspension straight on top of the gel, without touching the it. Make sure to deposit the cell suspension close to the gel and drop-by-drop to avoid bubbles getting trapped.

**h.** Once every well is seeded, transfer the plate into an incubator and incubate for 1 hour in cell culture conditions<sup>(4)</sup>.

**i.** After the cells have sedimented into the wells, gently add 2 mL of pre-warmed medium to the well<sup>(5)</sup>.

**j.** To change the medium, first remove the medium from the well and add 2mL of pre-warmed medium. To ensure that the medium is sufficiently replenished, the medium can be changed twice.

**k.** *Optional: When working with a target molecule, add the molecule dissolved in a solution at the step you want to study instead of the medium.*

**l.** *Optional: In order to harvest the spheroids from the microwells for further analysis, pipette medium vigorously straight over the gel using a 1mL pipette until the spheroids detach. Alternatively, spheroids can be detached enzymatically by add 0.5mL of Trypsin to each well and incubating for 2-3 minutes.*

## Notes

(1) The adhesive protein provided by 4DCell is 50 $\mu$ g/mL fibronectin. The concentration and the choice of the protein can be adjusted according to the cell type.

(2) To avoid going over the gel and distorting it, the plate can be tilted to transfer the solution in the seeding well to the macro well.

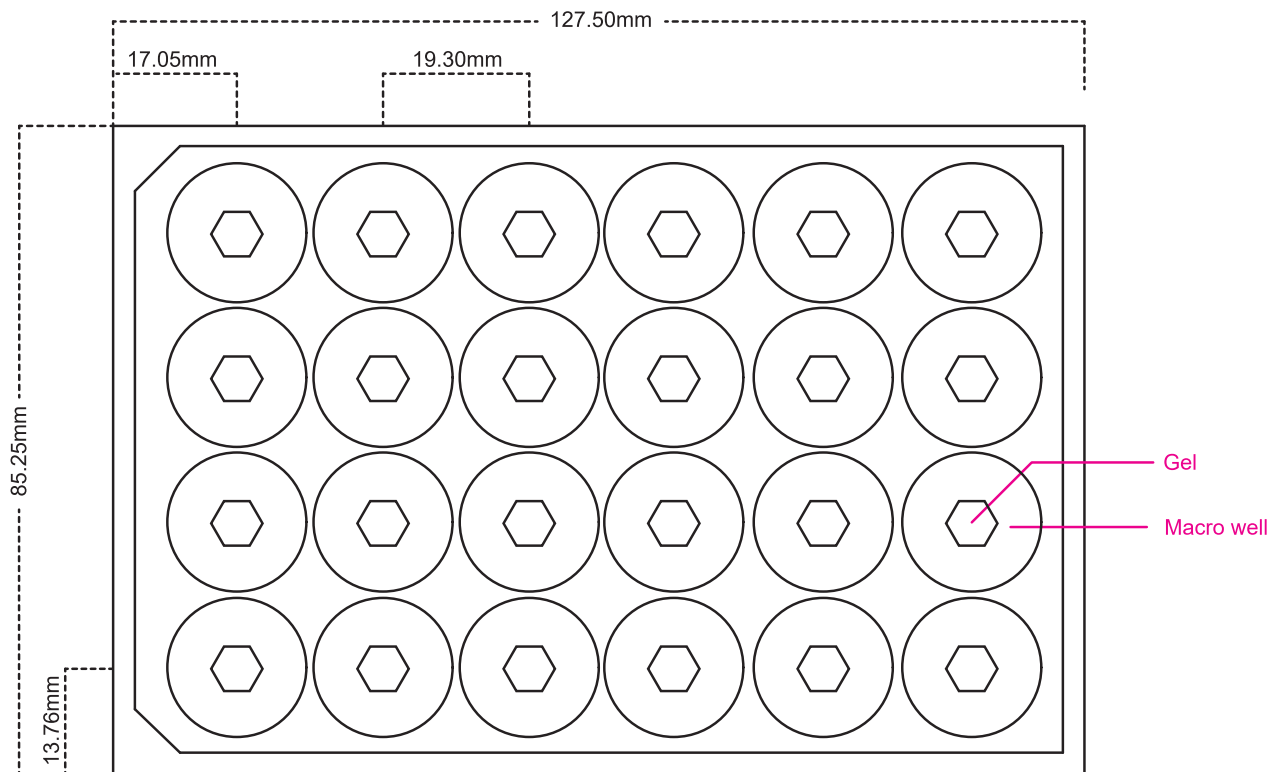
(3) The concentration used for 3000 MSCs per spheroid is 0.546x10<sup>6</sup> cells/mL. The concentration, and thus the number of cells per spheroid, can be changed according to the desired spheroid size or the cell type.

(4) This period of sedimentation has been optimized for MSCs. It may vary according to the cell type.

(5) The cells should have sedimented before adding more medium. The cells should be at the bottom of the seeding wells, which can be observed as round aggregates of cells separated from each other.

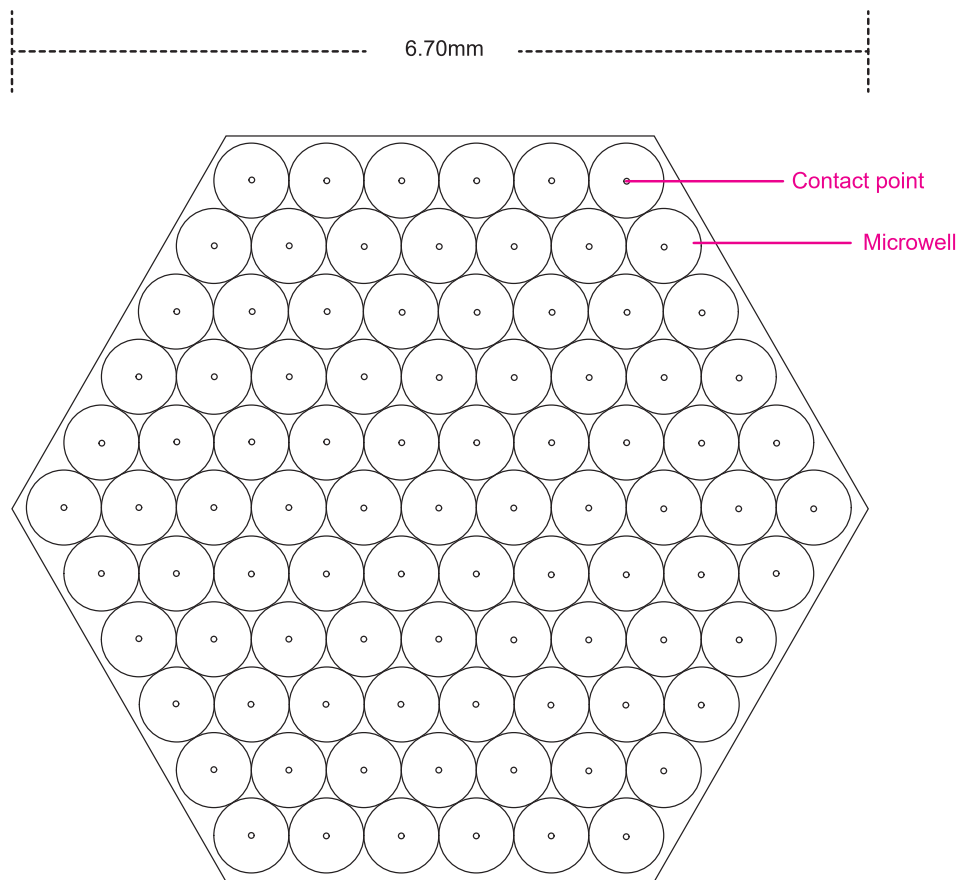
## Appendix

### 24-well plate

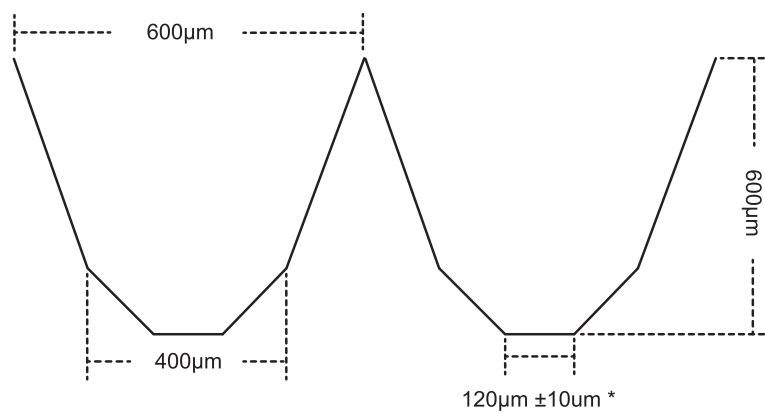


## Appendix

### Microstructured gel



### Cross-section microwell



\* Customizable