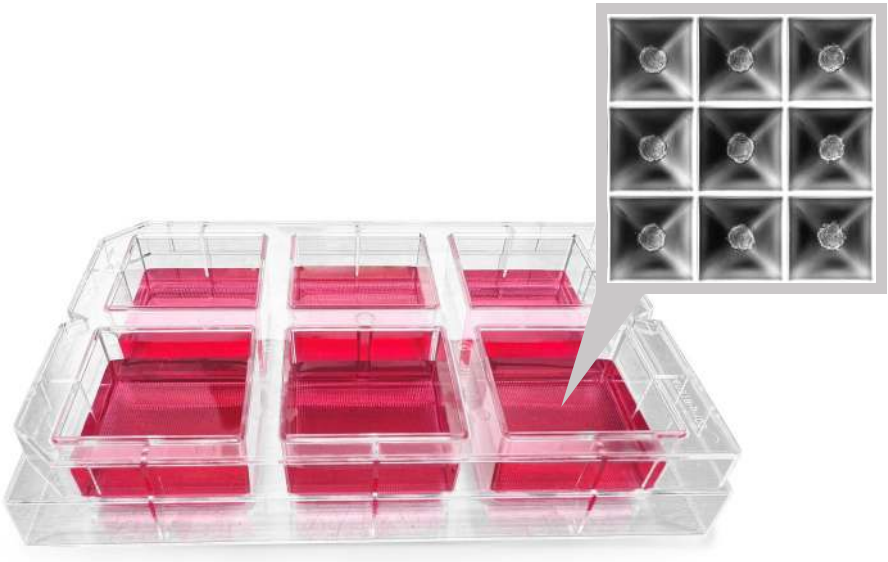
# SPHERICALPLATE 5D®

## Ecosystem for Regenerative Medicine



- Easy to use platform for spheroid formation
- Enabling standardized and uniformly sized spheroids
- Convenient upscaling without loss of spheroid quality

**1 Sphericalplate 5D**, 6 wells, 3’364 microwells each = 20’184 spheroids

## TURN PIPETTING INTO PUBLISHING

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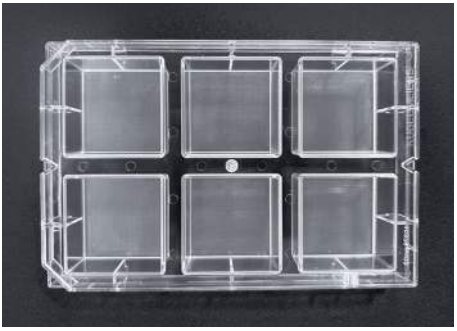
# Sphericalplate 5D manual in a nutshell

Step 1

## Preparation

Prepare functional well with 1 mL of medium



Step 2

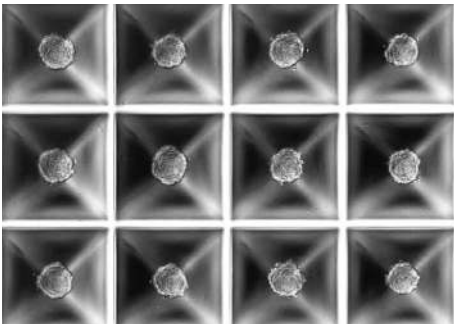
## Addition of cells

Add 1 mL of single cell suspension

Step 3

## Cultivation

Incubate



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EASY HANDLING

# Sphericalplate 5D - Directions for use

## Initial cell seeding

1. Before cell seeding, pre-wet the functional wells (microwells) of the Sphericalplate 5D using 2 mL rinsing medium. Rinsing fluid can be culture medium with or without serum supplement, or plain PBS. Do not allow the microwells to dry out.

   **Note:** Due to the applied coating, the medium usually flows regularly everywhere and the air bubbles are released by themselves. Depending on the medium used, some air bubbles can remain within the microwells. If so, they usually release either by light tapping of the Sphericalplate 5D or by centrifugation at 1000 x g for 1 min. Visual inspection by bright field microscopy is highly recommended to ensure that no bubbles remain trapped within microwells.

2. Calculate the desired number of cells per microwell and resuspend the cells considering they will be seeded in 1 mL medium per well. Pre-load the well with 1 mL of cell-free medium. Then add your cell suspension in another 1 mL of medium, for a total of 2 mL per well.

   Since cells travel by gravity into the microwells, make sure to generate an evenly distributed cell suspension in a short time. The better the cell suspension is mixed, the more regular the spheroids will be.

   **Note:** One functional well of the Sphericalplate 5D contains 3'364 microwells. The plate allows a wide range of different sizes of standardized spheroids. On average, for a spheroid to reach 100 µm diameter, 150-600 cells per microwell are needed. For fast-growing cells, it is recommended to seed fewer cells, i.e. 50 cells per microwell. To create large spheroids, it is feasible to load a larger quantity of cells per microwell, i.e. 1'500 cells per microwell.

   To obtain a uniform single-cell suspension without cell aggregation, the use of a cell strainer (e.g. 70 µm) is recommended before seeding. Tumor cells, for example, clump less if the cells are not agitated by hitting or shaking the flask while waiting to detach (e.g. during trypsinization).

3. After seeding, incubate according to the appropriate standard protocol. No further centrifugation is required.

## Medium change

4 After spheroid formation has occurred, carefully aspirate supernatant by placing the pipet just below the surface of the medium (away from spheroids) to avoid turbulence. The microwell height has been designed to retain the spheroids during the medium change, but care should be taken not to dislocate them.

**Note:** Pipetting must be very slow, otherwise a shock wave might arise, pushing spheroids out of their original microwell and displacing them from one microwell to another one. This should be monitored microscopically.

## Spheroid harvest

5 Tilt the plate at 20 to 30 degrees before entering the well with the pipet. Flush the well from top to bottom using a pipette and harvest the total amount of supernatant containing the spheroids into appropriate container for further analysis. In case of further cultivation of the spheroids within the plate, avoid tilting of the whole plate and perform the flushing procedure directly. Be aware that there might be a small loss with respect to harvest quantity; if needed, the well can be rinsed further with medium to harvest remaining spheroids.

## Various

**Plate specifications:** The Sphericalplate 5D is a 6 well plate of which wells marked A1-A3 and B1-B3 are loaded with 3'364 microwells each. A plate contains 20'184 standardized microwells in total.

**Culture conditions:** The culture conditions of your specific cells within the Sphericalplate 5D need to be determined individually. For example, oxygen tension within the medium is dependent on medium height. Spheroid size can reach critical sizes concerning oxygen tension in the spheroid core. Therefore, adjust the amount of medium to your cell metabolism. A final volume of 2 mL per well is a starting suggestion.

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