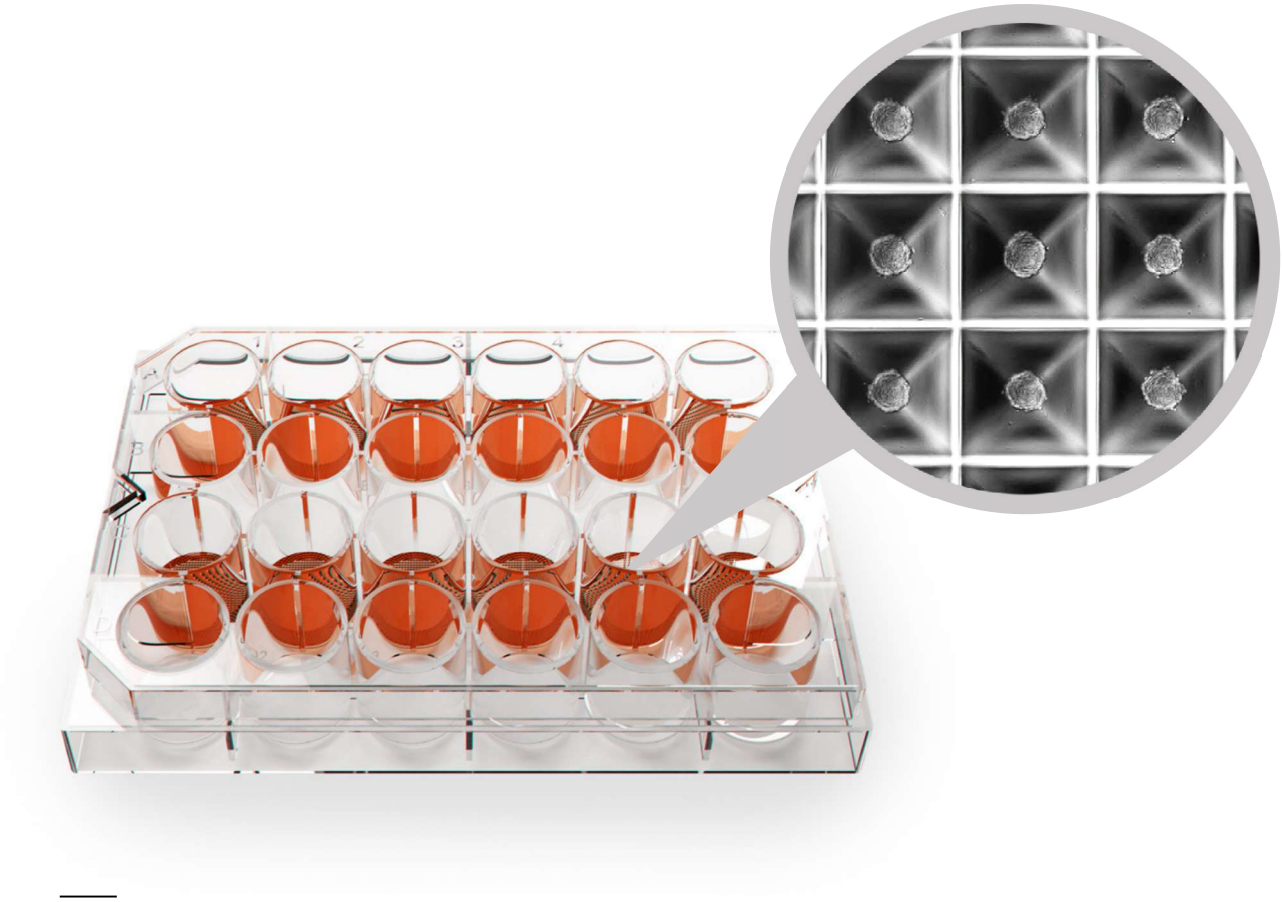
SPHERICALPLATE 5D®



—

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

1 Sphericalplate 5D, 12 wells, 750 microwells each = 9000 spheroids

TURN PIPETTING INTO PUBLISHING

#communicare

KUGELMEIERS®

Sphericalplate 5D SOP in a nutshell

Step 1

Preparation

Prepare functionalized well with 0.5 mL of medium



Step 2

Addition of cells

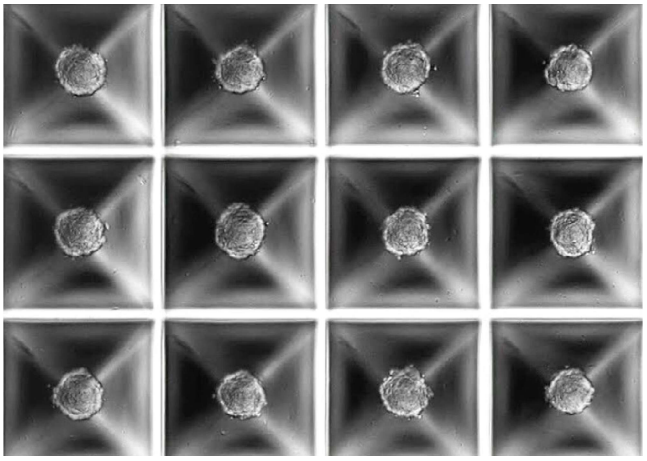
Add 0.5 mL of single cell suspension



Step 3

Cultivation

Incubate



EASY HANDLING

Sphericalplate 5D SOP extended

Initial cell seeding

1. Before cell seeding, pre-wet the functionalized wells (microwells) of the Sphericalplate 5D using 1 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 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 0.5 mL medium per well. Pre-load the well with 0.5 mL of cell-free medium. Then add your cell suspension in another 0.5 mL of medium, for a total of 1 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 functionalized well of the Sphericalplate 5D contains 750 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. 40 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 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, prevent the tilting of the whole plate and directly perform the flushing procedure. 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 24 well plate of which wells A1-A6 and C1-C6 (12 wells in total) are loaded with 750 microwells each. A plate contains 9'000 standardized microwells in total. The rows B1-B6 and D1-D6 can, if needed, be used for cultivation of the corresponding 2D cell culture.

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 1 mL per well is a starting suggestion.

Long-term cultivation: Depending on the incubation process (incl. humidity, volume and frequency of microscopic examination) evaporation across the plate can occur during long-term cultivation. In this case, an incorporation of an evaporation buffer (e.g. sterile PBS) using the not functionalized outer wells (B1-B6 and D1-D6) is recommended.

Kugelmeiers Ltd.
www.kugelmeiers.com

Bahnhofstrasse 40
CH-8703 Erlenbach

KUGELMEIERS®
ENABLING UNLIMITED CELL THERAPIES

FOR RESEARCH USE ONLY. NOT FOR CLINICAL DIAGNOSTIC OR THERAPEUTIC USE