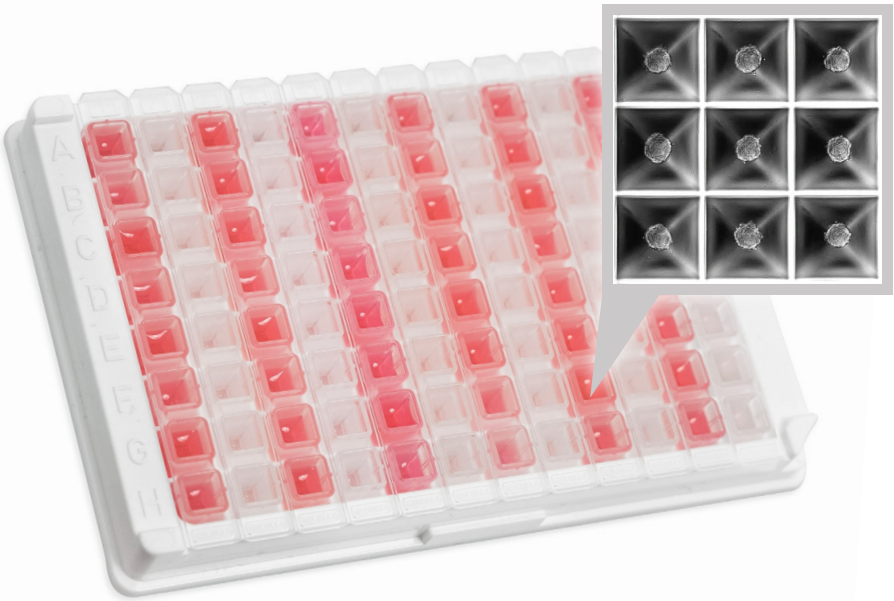
SPHERICALPLATE 5D®

Ecosystem for Cancer Spheroid Research



- Easy to use platform for spheroid formation
- Enabling standardized and uniformly sized spheroids
- Flexibility in experimental setup design

1 Sphericalplate 5D, 8 wells per strip, 25 microwells each
2400 spheroids per plate (12 strips mount on 96-well frame)

TURN PIPETTING INTO PUBLISHING

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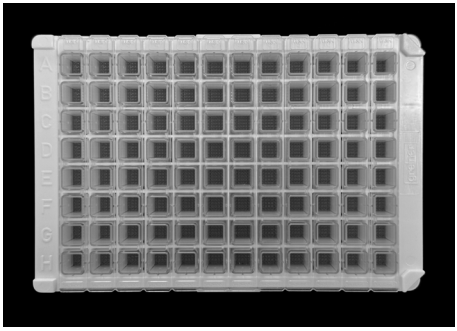
Sphericalplate 5D manual in a nutshell

Step 1

Preparation

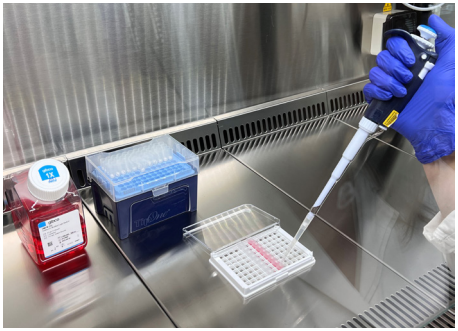
Prepare functional well with 0.1 mL of medium



Step 2

Addition of cells

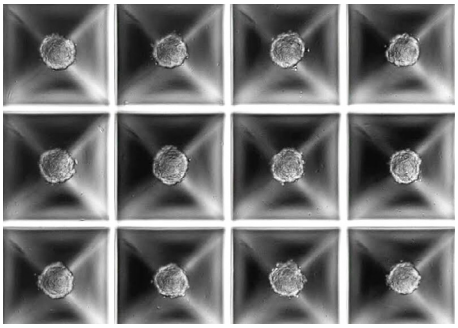
Add 0.1 mL of single cell suspension



Step 3

Cultivation

Incubate



EASY HANDLING

Sphericalplate 5D - Directions for use

Initial cell seeding

1. Before cell seeding, pre-wet the functional wells (microwells) of the Sphericalplate 5D stripusing 0.1 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 strip 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 at least 0.2 mL medium 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 strip contains 25 microwells. The strip 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 fastgrowing 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. 1500 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. For the full spheroid harvest from platform, tilt the frame 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. For partial spheroid harvest, take the strip of interest individually out of the frame and follow the procedure as described above. 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 strip is a 96-well format consist of 12 individual strips. Each strip has 8 wells with 25 round-bottomed microwells. A whole 96-well platform contains 2400 standardized microwells in total.

Culture conditions: The culture conditions of your specific cells within the Sphericalplate 5D strip need to be determined individually. For instance, 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 0.2 mL per well is a starting suggestion.

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