SPHERICALPLATE 5D®

3D cell culture rEvolution®

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CLINICAL GRADE, REGULAR 3D CELL CLUSTERS

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

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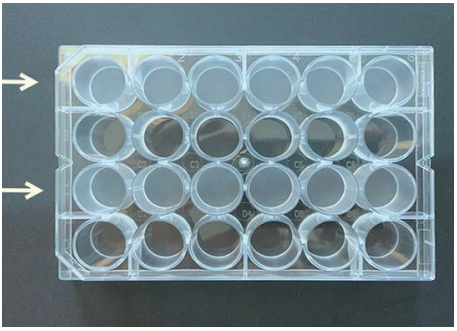
Sphericalplate 5D SOP in a nutshell

Step 1

Preparation

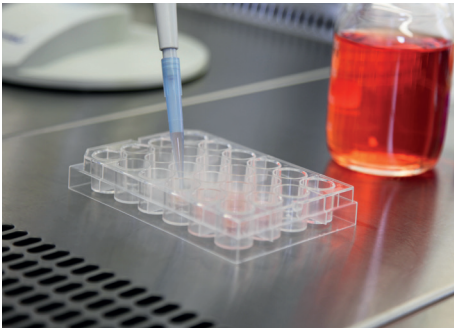
Prepare functionalized well with 0.5 mL of medium



Step 2

Addition of cells

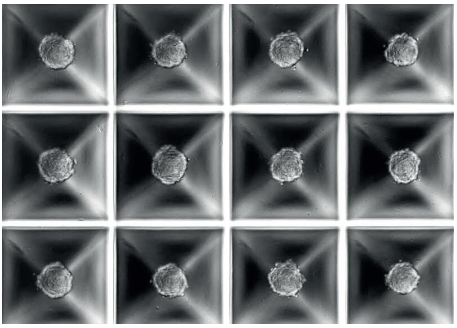
Add 0.5 mL of single cell suspension



Step 3

Cultivation

Incubate



EASY HANDLING

Sphericalplate 5D SOP extended

Initial cell seeding

1. Pre-wet the Sphericalplate 5D with medium by rinsing the functionalized wells with 1 mL of complete cell culture medium or PBS. Do not allow the wells to dry.

   Note: Due to the nanocoating, 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. Check that no bubbles remain under the microscope.

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. The better the cell suspension is mixed, the more regular the spheroids will be. No further centrifugation is required.

   Note: One functionalized well of the Sphericalplate 5D contains 750 microwells. The plate allows a wide range of different sizes of standardized clusters depends on the cell type. On average, for a cluster 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 very large clusters it is feasible to load a larger quantity of cells per well, i.e. 2'000 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.

Medium change

4. After the first cluster formation has happened, carefully aspirate supernatant by placing the pipet just below the surface of the medium to avoid turbulence. The microwell height has been designed to retain the clusters during the change, but care should be taken.

5. Be very careful when dispersing fresh medium back to the well. It can cause a "wave-like" effect upon the cells and single clusters could be washed out and hop from one microwell to another one. This should be monitored microscopically.

Easy cell cluster harvest

6. Tilt the plate before entering the well with the pipet. Flush the well by swirling the plate followed by the harvesting of cluster suspension. In case of further cultivation of the clusters within the plate prevent the tilting of the whole plate and directly perform the “swirling” 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 clusters.

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 9000 standardized microwells in total. The rows B1-B6 and D1-D6 can, if necessary, 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. Cluster size can reach critical sizes concerning oxygen tension in the cluster 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.