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Using IMAPlate™ 5RC96 as a "hanging drop" cell culture device for 3D cell culture

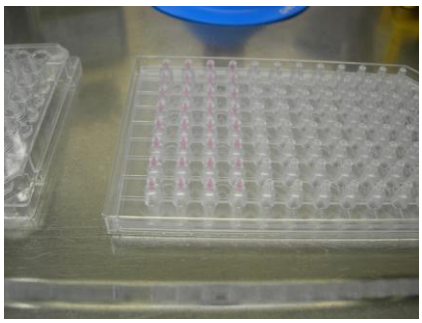
It has been reported that the physiology of cells can be quite different when cells are cultured in typical two-dimensional (2D) environments and three-dimensional (3D) environments. Drug candidates that show promise in conventional monolayer cell cultures often fail in later stages of drug development. 3D spheroids resemble tissues in vivo and their gene expression is much closer to clinical expression profile than in 2D. Therefore, it has been increasing to use 3D spheroids instead of 2D monolayer cells for the studies of proliferation, apoptosis, differentiation, gene regulation and metabolism in a mimic environment in living things. In order to eliminate ineffective drug candidates in the early process, using 3D spheroids for compound screen becomes more popular recently. Although 3D spheroids can easily be prepared with a hanging drop cell culture technique, there have been challenges such as to generate large quantity of spheroids, to change cell culture medium for each hanging drop and to transfer each single spheroid to the individual well of a 96- or 384-well plate.

The unique features of IMAPlate™ 5RC96 make it a user friendly "hanging drop" cell culture device for 3D spheroid preparation. Besides it can meet the mentioned challenges, IMAPlate™ 5RC96 has following advantages as well. 1) Cell suspension can simultaneously be added to IMAPlate™ 5RC96 in seconds by touch loading. 2) Cells can readily form spheroids in the small diameter capillarity chamber. 3) A shaker can also be used to facilitate the formation of spheroids since culture medium in IMAPlate™ 5RC96 does not drop out. 4) Size of spheroid can easily be estimated under the microscope. 5) Cells or spheroids will not be sucked up during cell culture medium change. 6) Preparation of spheroids and compound testing can be done in the same IMAPlate™ 5RC96. 7) Spheroids can very gently be transferred to any 96-well plate or a 384-well plate just by placing IMAPlate™ 5RC96 to the 96- and 384-well plate which contains culture/testing medium in the wells. 8) Spheroids can directly be spotted onto a microscope slide from IMAPlate™ 5RC96 for histological analysis.

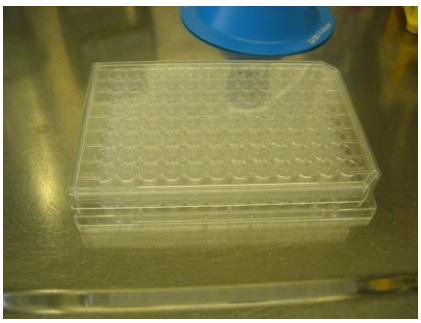
Preparation of spheroids for U87, LN319 and HEK cells

A. Seeding cells to IMAPlate™ 5RC96

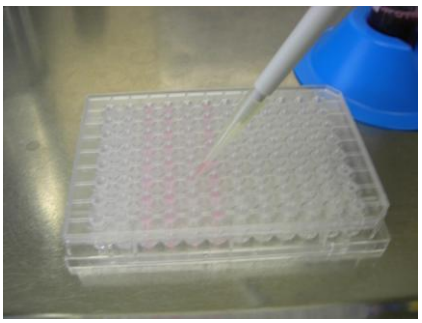
1) Prepare single cell suspension from 80-95% confluent monolayer cell culture and make the cell concentration to your experimental requirement. (Seeding 1,000-5,000 cells to the capillarity chamber will form a spheroid with a size around 0.05-0.3mm in diameter in 2 to 4 days.)



2) Turn a sterile IMAPlate™ 5RC96 upside down and pipette 6µl of single cell suspension to the bottom open of the capillarity chamber of the "well". (*For large quantity of spheroids preparation, touch-loading is recommended.*)



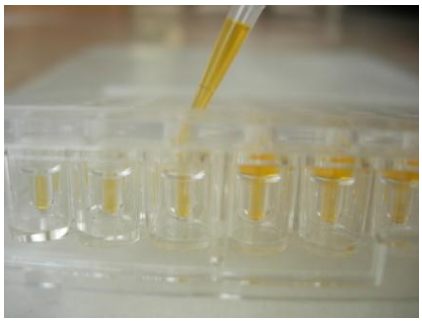
3) Place the IMAPlate™ 5RC96 on a sterile 96-well plate that serves as a stand. Let the cells to settle down for 2-5 minutes with a plate cover on the top. (*Please be aware of the fast evaporation in laminar flow workstation. Excessive loss of cell culture medium may generate an air gap in the capillarity chamber when culture medium is added to the reservoir in next step 4.*)



4) Pipette 25-35µl of cell culture medium to each corresponding reservoir of the "well" where the capillarity chamber containing cells. In order to reduce the evaporation of the culture medium, pipette 40-50µl of sterile ddH_2O to the rest of reservoirs. (*It is recommended to reserve the outmost "wells" for addition of sterile ddH_2O if not all the "wells" are used.*)

5) Put the IMAPlate™ 5RC96 along with the 96-well plate and the cover in cell culture incubator.

B. Changing fresh culture medium every two to three days (depending on the culture conditions and the evaporation rate)

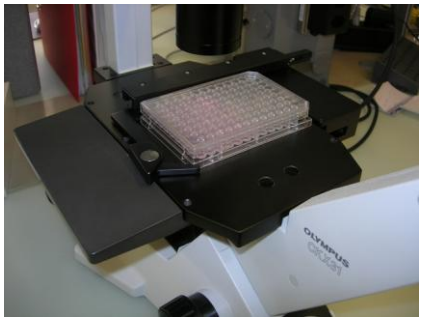


1) Remove the plate cover, insert the pipette tip along the wall till to the end of the reservoir and suck out the old culture medium in the reservoirs completely.

2) Pipette 25-35µl of fresh culture medium to the reservoirs immediately.

3) Cover the IMAPlate™ 5RC96 and put back in cell culture incubator.

C. Transferring spheroids to 96-well plate for assays



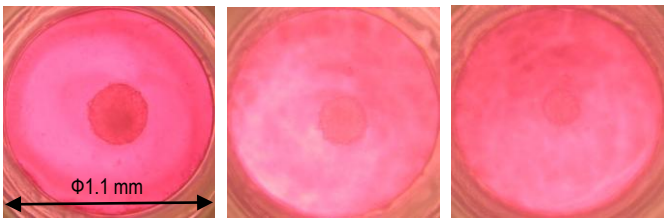
1) Check spheroid in the capillarity chamber of the IMAPlate™ 5RC96 under an inverted microscope with a 10X objective lens. Estimate the size by comparing the spheroid to the capillarity chamber, which is 1.1mm in diameter.

2) When the spheroids have the right size that meets your need. Prepare an assay plate by addition of 100 µl of assay medium to correspondent wells of the 96-well plate.

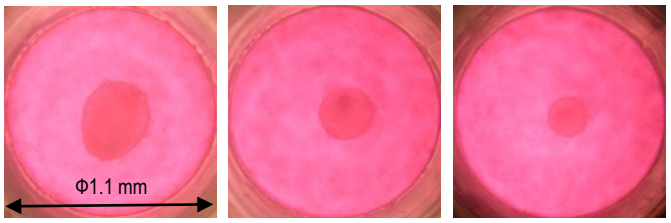
3) Remove the plate cover and suck out the culture medium in the reservoir completely. (Omit this step if there are no influences on the assay when small amount of culture medium flows into the well from IMAPlate™ 5RC96.)

4) Place the IMAPlate™ 5RC96 on the assay plate and let the bottom open of the capillarity chamber to contact the assay medium for a few seconds to allow the spheroid to go down to the bottom of the wells under the gravity. Remove the IMAPlate™ 5RC96 and then cover the 96-well plate.

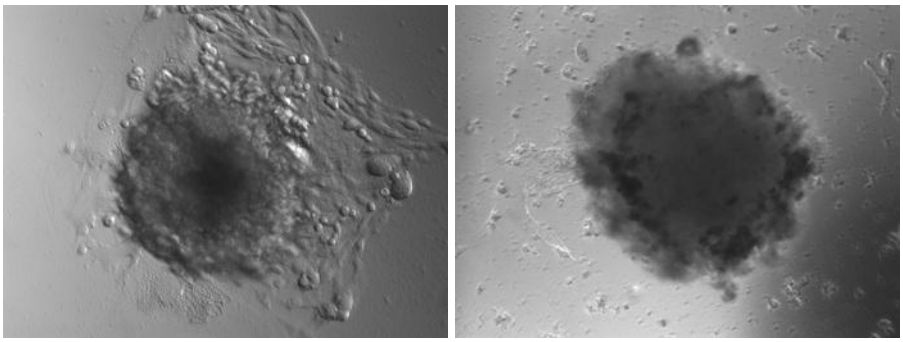
6) Perform the assay in the 96-well plate according to the procedure of the assay.



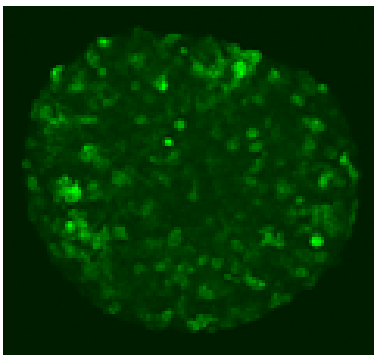
2 days after U87 cells seeded in IMAPlate™ 5RC96
(cell seeding number is 5000, 2500 and 1000 from right to left)



4 days after U87 cells seeded in IMAPlate™ 5RC96
(cell seeding number is 5000, 2500 and 1000 from right to left)



Response of spheroid to test compounds
(2 days after spheroids of LN319 transferred to a 96-well assay plate)



Spheroid prepared from HEK cells
expressing green fluorescent protein