Frequently Asked Questions Regarding the Akura™ 3D SureKit

A detailed protocol for production of hepatocyte spheroids is provided in this product manual. Below are answers to some frequently asked questions to help get you started.

Q: What is the optimal volume per well in the Akura™ 96 Plate?

A: To achieve optimal volume per well, gently deliver 70 µl (pipetting speed < 10 µl/sec) of cell suspension into each well of the Akura™ 96 Plate by placing the pipette tips near, but not touching, the bottom of the wells.

Important - For spheroids with uniform size and cell composition it is essential to assure a homogeneous distribution of the cells by gently pipetting up and down prior to seeding into the Akura™ 96 Plate.

Q: Why do you recommend centrifuging the Akura™ 96 Plate after cell seeding?

A: We recommend to briefly centrifuge the plate after cell seeding to remove any air bubbles and to force the cells to the bottom of the well in order to promote cell-aggregation and spheroid formation.

For that, place the lid on the plate and spin in a microtiter-plate centrifuge for 2 minutes at 200 RCF. Afterwards, incubate the plate in a humidified CO^2 incubator at 37 °C for 2-5 days.

Q: Are there enough cells in the vial to aggregate larger tissues?

A: Yes, the vial contains sufficient cell numbers to aggregate large spheroids (up to 2500 cells/spheroid)

Q: How do I exchange the medium in the Akura™ 96 Plate without disturbing or losing the spheroids?

A: To prevent spheroid/organoid loss during the exchange of media, the SureXchange™ ledge serves as an anchoring point for the pipette tip. Just place the tip at the ledge of the well, see figure below, and remove the medium at low pipetting speed (>30 µl/sec). A minimal volume of ~ 5-7 µl will remain in the well.

Then, add 70 µl of fresh medium by placing the pipette tip at the ledge, use dispensing rate <50 µl/sec.

Important - when using automated liquid handling devices, an off-center alignment of the vertical pipette tip will achieve the same effect.

Q: What is the best way to prevent evaporation in the outer wells of my plates?

A: Evaporation in the outer (perimeter) rows of wells is a phenomenon common to most low-volume culture platforms, and thus requires careful attention to maintaining proper humidity control. If not controlled, pronounced evaporation can result in concentration or precipitation of media components (serum, salt) that can impact spheroid formation or health, and can alter the effective concentration of a compound/additive in the medium over the course of a long-term experiment. To provide maximum humidity control when using the Akura™ Plates, we recommend the following:

Use an incubator with good humidity control (>95% of rel. humidity), and exercise best practice in maintaining and minimizing loss of humidity (e.g., minimize incubator door opening and closing).

For culture in the Akura™ 96 Plate, at least 50-70 µl of medium in each well is recommended and can be increased to a maximum of 80 µl if incubator humidity control is a persistent issue. Medium exchange frequency can also be increased to every other day or daily if conditions dictate.

We recommend the use of the InSphero Incubox™ to reduce edge effects when performing long-term culture with low-frequency medium exchange. The Incubox™ is available in shop.insphero.com.

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If you have more questions, please refer to the FAQs section on shop.insphero.com

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