Frequently Asked Questions Regarding the Akura™ 96 Spheroid Microplate

A detailed protocol for production of spheroids in the Akura™ 96 Spheroid Microplate is provided in the product manual. Below are answers to some frequently asked questions to help get you started.

Q: What improvements did you make to the new Akura™ 96 Plate?

A:

Improved optical properties:

- COP (Cyclo-Olefin Polymer, 92% transparency 400-800 nm) as plate material instead of Polystyrene.
- Thinner well bottom of 0.8 mm, before 1.3 mm.
- Reduced skirt height of 0.4 mm. High NA objectives (e.g., 20X and 40X) may be used to image the outer wells of the plate

Automation friendly:

- Excellent planarity across plate (below 80 µm) for reliable spheroid transfer and precise medium exchange

Less evaporation:

- Optimized distance (200 µm) between customized low-evaporation lid and plate reduces evaporation in outer and edge wells

Standard SLAS plate height:

- 14.35 mm plate height instead of 11.48 mm
- Maximum volume 280 µl instead of 170 µl

Q: Why do you recommend pre-wetting of the wells prior to cell seeding?

A: Pre-wetting the wells of the Akura™ 96 Plate is required prior seeding to prevent inclusion of air-bubbles. For that, apply 40 µl of your cell medium to each well by placing the tips far into the wells. Remove the pre-wetting solution by placing the tip at the ledge of the upper cavity of the well. Aspirate medium until is completely removed from each well. A negligible amount (< 5-7 µl) may remain in the bottom of the chamber.

Q: Can I create spheroids from any cell type?

A: Not all cells aggregate to spheroids. First, it highly depends on the architecture, function, and morphology of the tissue/organ of which they are isolated from and how the isolation affected the cells. Further, we see variations in successful aggregation between donors for the same cell type. Plateable cells, in general, have a good chance to form spheroids. To achieve optimal spheroid formation, it may be necessary to modify the aggregation conditions such as modifying the cell concentration or media composition, or by the addition of supporting cell types (e.g., matrix secreting cells) or supplements (e.g., ECM). For new cell types we recommend trying a variety of aggregation conditions.

Q: Could you recommend a cell concentration for my cell suspension for the generating of spheroids/organoids?

A: For long-term growth profiling, we recommend starting with low cell numbers (250 – 500 cells per well of 70 µl). If use of non-proliferating cells or rapid production of larger spheroids are required, start with higher numbers (from 2500+ cells per 70 µl). Generally, we recommend trying different concentrations for defining your optimal range when using new cell types.

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

A: To achieve optimal conditions, 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 Spheroid Microplate 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 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 250 RCF. Afterwards, incubate the plate in a humidified CO_2 incubator at 37 °C for 2-5 days.

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

A: To prevent spheroid/organoid loss during the exchange of media, the SureXchange™ ledge at the inside wall of each well 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:

1. 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).
2. For culture in the Akura™ 96 Spheroid Microplate, 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.
3. We recommend the use of the InSphero Incubox™ (CS-AH11) (Figure 10) to reduce edge effects when performing long-term culture with low-frequency medium exchange. The InSphero Incubox™ is available on shop.insphero.com.

Figure 10: InSphero Incubox™

Q: What do I need to consider when using the plates for imaging?

A: In order to achieve optimal results, a few relatively simple changes need to be made by a knowledgeable instrument operator. By adhering to the suggestions below, the Akura™ 96 Plate can be used successfully with nearly all high content imaging platforms:

- Due to the tapered well bottom and the 0.8 mm bottom thickness, the creation of a new 96 well plate definition (a.k.a. form factor) may be required for optimal imaging performance (use the specifications provided in our online store as the starting point for the new plate definition).
- The non-continuous well bottoms and 0.8 mm bottom thickness may necessitate the use of an extended autofocus range to ensure accurate focus across the entire plate.
- If image acquisition through the entire Z height of the spheroids is required, the working distance of the selected objective must be equal to the bottom thickness (0.8 mm) plus the Z height of your specimen.
- Objectives with correction collars should set for a 0.8 mm bottom thickness.

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

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