Frequently Asked Questions Regarding the Akura™ PLUS Hanging Drop System

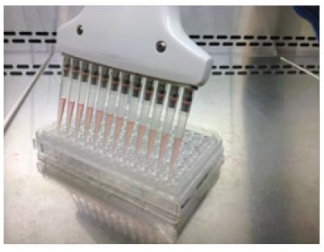
A detailed protocol for production and transfer of spheroids in the Akura™ PLUS Hanging Drop System is provided in the product manual. Below are answers to some frequently asked questions to help get you started.

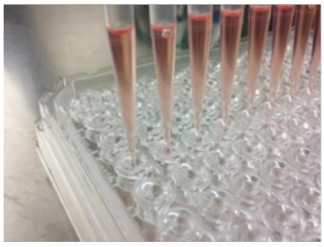
Q: Could you recommend a cell concentration for my cell suspension for the generating of spheroids?

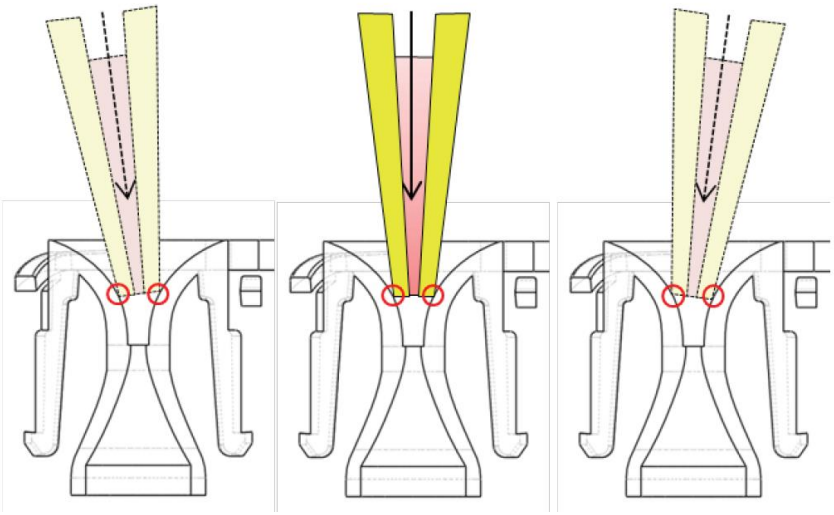
A: For long-term growth profiling, we recommend starting with low cell numbers (250 – 500 cells per drop of 40 µl). If use of non-proliferating cells or rapid production of larger spheroids are required, start with higher numbers (from 2500+ cells per 40 µl). Generally, we recommend trying different concentrations for defining your optimal range when using new cell types.

Q: What is the SureDrop™ inlet?

A: InSphero's Akura™ PLUS Hanging Drop Plate features the patent pending SureDrop™ microtechnology, which allows for precise dispensing into and aspirating from hanging drops. As the drop volume corresponds to the seeding cell number, spheroids produced in Akura™ PLUS Hanging Drop Plates display outstanding size consistency, with variation in diameter of 5% and less across an entire 96-well plate. To assure the SureDrop™ seal, it is important that the pipette tips make sufficient contact with the well surface to assure complete liquid transfer and uniform drop formation. The spring-loaded wells elasticity adjusts to ensure contact is maintained when downward pressure is applied during pipetting.







Q: What is the optimal volume per drop in the Akura™ PLUS Hanging Drop Plate?

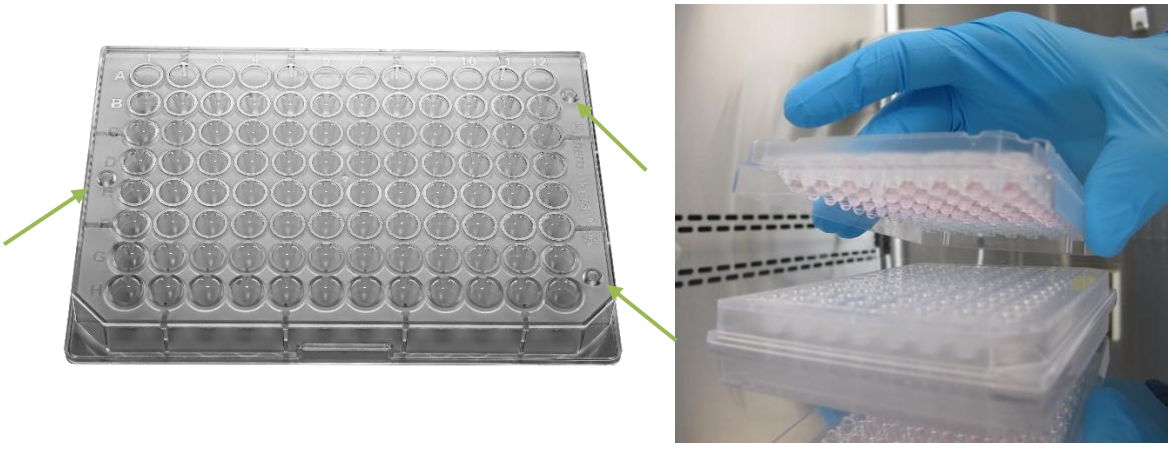
A: To achieve an optimal and stable hanging drop, gently deliver 40 µl (pipetting speed < 50 µl/sec) of cell suspension into each well of the Akura™ PLUS Hanging Drop Plate. Important: To generate spheroids that are uniform in size and cell composition it is essential to assure a homogeneous distribution of the cells by gently pipetting up and down prior to seeding into the Akura™ PLUS Hanging Drop Plate.

Q: Why do you recommend pre-wetting Akura™ 96 Plate wells prior to spheroids transfer?

A: Pre-wetting the wells of the Akura™ 96 Plate is required prior to transfer to prevent inclusion of air-bubbles. For that, apply 40 µl of aggregation 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: How do you transfer spheroids into the receiver (Akura™ 96) Plate?

A: Place the frame with stripes of the Akura™ PLUS Plate onto the Akura™ 96 Plate by positioning the three pins into the corresponding holes on the top surface of the Akura™ 96 Plate. The drops under the Akura™ PLUS Plate will then be perfectly aligned with the wells of the Akura™ 96 Plate underneath. Using a slow pipetting speed (≤ 10 µl /sec), add 70 µl of medium or buffer through the inlet of the Akura™ PLUS Hanging Drop Plate wells. Make sure that the pipette tip forms a tight contact with the well inlets.



1. Merge the Akura™ 96 Plate with the Akura™ PLUS Hanging Drop Plate.
2. Specific pins and pinholes allow for easy connection of the 2 plates.
3. Additional ‘pins’ provide proper distance of the plates.

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 maintain proper humidity control. Evaporation in the outer rows of hanging drops (Akura™ PLUS Plate) or wells of the Akura™ 96 Plate although infrequent when following the recommendations below, is a possibility. If not controlled, pronounced evaporation can result in concentration or precipitation of media components (e.g., 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™ PLUS Hanging Drop System, 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).
- During microtissue formation in the Akura™ PLUS Hanging Drop Plate, always use the supplied humidifier pad in the base plate, thoroughly soaking it with sterile 0.5x diluted PBS. This is key to maintaining proper humidity in the Akura™ PLUS Plate during microtissue maturation in the hanging drop.
- For incubators with poor humidity control, hypotonic buffer solutions (e.g., 0.2× PBS) may be applied to the humidifier pad.
- 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™ (InSphero CS-AH11) (Figure 20) to reduce edge effects when performing long-term studies with low-frequency medium exchanges. The InSphero Incubox™ is available on shop.insphero.com

Figure 20: InSphero Incubox™

Q: Why do my cells not form spheroids?

A: There are several reasons why a particular cell line, cell type, or co-culture may not re-aggregate and form a spheroid. Pre-treatment/harvesting conditions, cell seeding density (or ratio in co-culture models), media formulation, incubation time, media evaporation, and many other factors can impact the success or failure of microtissue production and culture.

Many tumor cell lines are known to readily form microtissues in ultra-low attachment (ULA) treated plates, and we recommend our Akura™ 96 Plates CS-PB15 as a starting point if the literature suggests this is the case for your cell line of interest, or if you are unsure.

For primary cells, more complex co-cultures, or cell lines that fail to form spheroids in ULA plates, we recommend the Akura™ PLUS Hanging Drop System (CS-PF24). Some cells may require co-culture with fibroblasts or supplemental growth factors in order to form dense, spheroids, or to establish and maintain an organotypic phenotype.

Q: What is the best way to image spheroids in the hanging drop?

A: Spheroid formation, appearance and growth profiles can be assessed using an inverted brightfield microscope. A long-working-distance objective (LWDO), preferentially of 10x magnification, is required for proper imaging. Depending on the minimal gap (D1) between the objective plane and the microscope stage, the specifications of the objective should include a working distance of minimally 11.5 mm + D1. The included humidifier pad has a rectangular opening in the center to allow easy visualization of hanging drops in the most central wells. If microscopic assessment of all 96 wells is desired, simply transfer the Hanging Drop Plate to an empty, sterile base plate for imaging, then return to the humidified base plate prior to returning to the incubator.

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

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