Akura™ PLUS Hanging Drop System
Quick Start Guide

Thank you for choosing InSphero's Akura™ PLUS for your 3D cell culture experiments. This Quick Start Guide contains important information to get you started immediately. For detailed instructions please refer to the Product Manual and additional resources on shop.insphero.com.

Akura™ PLUS Hanging Drop System Components

A. Akura™ PLUS Plate (frame with 12x8 well strips) + humidifier pads (in bags with tweezer)

B. Transparent lid

C. Bottom plate with reservoir

D. Akura™ 96 well plate

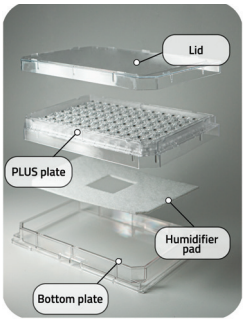

Figure 1. Akura™ PLUS system components.

Generating Spheroids or Organoids

1. **Preparation:** Prepare a reservoir with 20ml 0.5x PBS, remove one of the humidifier pads using the tweezer and place it in the reservoir. Wait approx. 5 min until the pad is completely soaked with PBS and place it in the bottom plate of the Akura™ PLUS Plate.

2. **Cell seeding:** Count the cells and prepare a cell suspension for seeding, using a final volume per drop of 40µl. For long-term growth profiling start with low cell numbers (250 – 500 cells per drop). For non-proliferating cells or rapid production of larger spheroids start with higher numbers (from 2,500 cells per drop). Please try different concentrations for defining your optimal range.

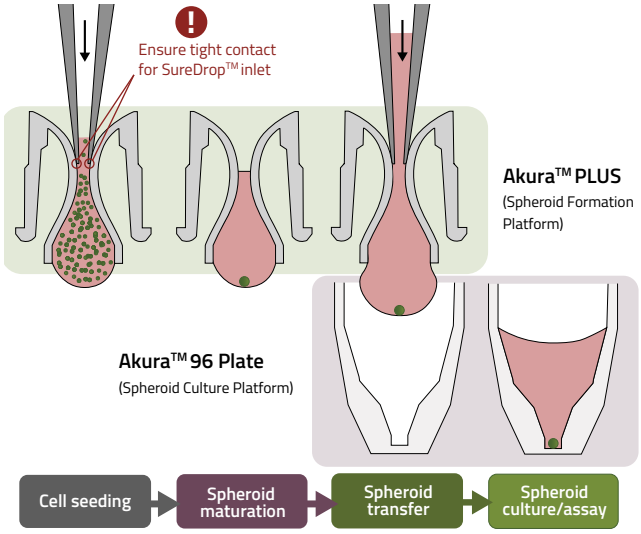

Figure 2: Delivering cell suspensions and generating spheroids in Akura™ PLUS Hanging Drop System.

Important: *To generate spheroids with uniform size and cell composition, it is essential to assure a homogeneous distribution of cells by gently pipetting up and down prior to seeding.*

3. Gently deliver 40µl of cell suspension into each well of the Akura™ PLUS Plate. Make a tight contact between the pipette tip and the well inlet by applying a slight pressure to form the SureDrop™ seal (Fig. 2)
4. Place the lid on the Akura™ PLUS Plate and place it in a humidified CO_2 incubator at 37°C.
5. Assess spheroid formation regularly. After 4 days in culture most cell types re-aggregate and form a compact spheroid.

Transfering 3D Spheroids or Organoids

For long-term cultivation and assays, transfer of the spheroids from the Akura™ PLUS Plate to the Akura™ 96 Spheroid Microplate is required.

Important: *In order to prevent inclusion of air bubbles, is is recommended to pre-wet the wells of the Akura™ 96 Plate. Apply 40µl of cell line medium in each well. Gently pipette the medium up and down and aspirate the pre-wetting medium from the plate just prior to seeding.*

1. Place 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 plate underneath.
2. Slowly (<10 µl/sec) add 70µl of medium through the inlet of the Akura™ PLUS Plate wells. The pipette tips should be in direct contact with the well inlets by simultaneously applying a subtle pressure with the pipette. The drops will fall into the Akura™ 96 Plate.
3. Sedimentation spin: It is recommended to briefly centrifuge the plate for 2 minutes at 250 RCF to force all tissues to the bottom of the cavity and to remove air bubbles.
4. To assure defined medium volumes in the wells, replace the solution in the wells by aspiration and addition of 70µl of fresh medium.

Medium Exchange in the Akura™ 96 Spheroid Microplate

1. Place the pipette tip at the ledge of the well.
2. Remove the medium at low pipetting speed (<30 µl/sec) by aspirating an excess of volume. A minimal volume of ~5-7 µl medium will remain in the well.
3. Add 70µl of fresh medium by placing the pipette tip at the ledge. Use a dispensing rate <50 µl/sec.
4. Place the lid on the Akura™ 96 Plate and place it in a humidified CO_2 incubator at 37°C.

For detailed information, please refer the Akura™ PLUS System Product Manual.

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

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