Akura™ 3D SureKit
Quick Start Guide

Thank you for choosing InSphero's Akura™ 3D SureKit 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.

What's in the box?

A. 3 x Akura™ 96 Spheroid Microplates

B. 1 x Akura™ Tilting Stand

C. Primary human hepatocytes,
cryopreserved (> 1 million per vial)

D. 40 ml Aggregation base medium stored at 2-8°C

E. 12.5 ml Aggregation supplement stored at <-15°C

F. 200 ml Maintenance base medium stored at 2-8°C

G. 10 ml Maintenance supplement stored at <-15°C

Protocol for generating 3D primary human hepatocyte spheroids

Upon Arrival

IMPORTANT: Open pouch and transfer cryo vial with primary hepatocytes to liquid nitrogen immediately.

Preparation

1. Prior to seeding, pre-warm the cell **Aggregation Base Medium**.
2. Thaw the **Aggregation Medium Supplement** in a water bath set to 37°C.
3. Wipe the pre-warmed Aggregation Medium bottle and Aggregation Supplement tube with 70% EtOH and transfer to sterile working area.
4. Pipet the contents of the **Aggregation Medium Supplement** to the **Aggregation Base Medium** (now **Aggregation Medium**) and mix.
5. Wipe the Akura™ Plate bag with 70% EtOH before opening.

Thawing

1. Remove vial with hepatocytes from cryo storage and thaw rapidly in a 37°C waterbath until only a small ice chip remains in the center of the vial.
2. Immediately wipe the vial with 70% EtOH and transfer it to the sterile working area.
3. Aspirate 4 ml of **Aggregation Medium** in a 5 ml serological pipet (if available use a wide bore pipet).
4. Then slowly aspirate thawed cell suspension.
5. Dispense 1.5 ml of this 4.5 ml into a 15 ml Falcon tube.
6. Wash the cryo-vial by adding 1.5 ml from the suspension in the pipet to the vial. Re-aspirate the 1.5 ml into the pipet. Transfer 1.5 ml to the Falcon tube (point 5).

7. Repeat step 7 with the remaining volume in the pipet.
8. Add additional 8 ml of **Aggregation Medium** to the Falcon tube (point 5).
9. Gently tilt the Falcon tube containing the cell suspensions 5 times.
10. Centrifuge Falcon tube at **80 RCF for 5 minutes.**

For detailed information, please refer to the Akura™ 3D SureKit Product Manual.

Hepatocyte seeding

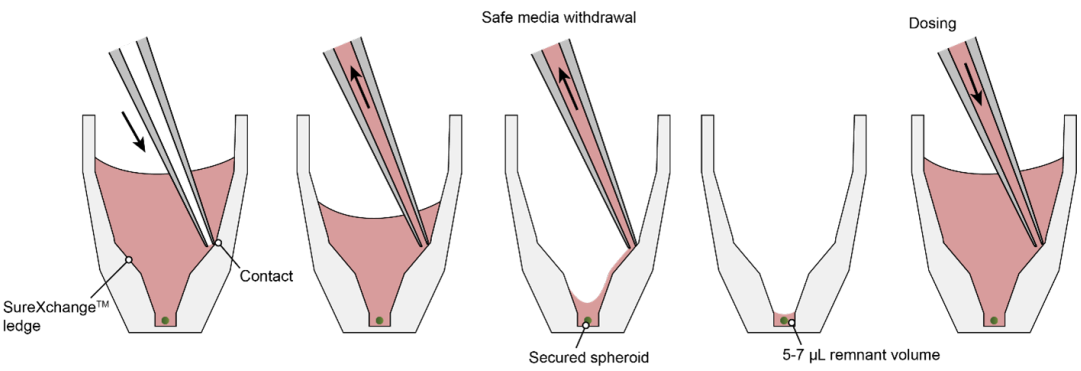
1. Carefully remove all the supernatant.
2. Dispense 5 ml of the **Aggregation Medium** to cells.
3. Resuspend pellet carefully by dislodging it with a 10 ml serological pipet (if available use a wide bore pipet) and very gently aspirate and dispense the suspension 2-3 times.
4. Determine cell density per milliliter by counting viable cells using a cell counter or a Neubauer counting chamber.
5. Prepare a cell suspension for seeding, using a final volume per well of 70 µl. Recommended cell concentration: **1150** cells per 70 µl.

IMPORTANT: For uniformity of spheroids, it is essential to assure a homogeneous distribution of the cell suspension by gently pipetting up and down the cell suspension in the reservoir prior to seeding into the Akura™ 96 Plate.

6. Gently (≤10 µl/sec) add 70 µl of the cell suspension per well to the Akura™ Plates by placing the pipette tips far into the wells.
7. Place the lids on the Akura™ 96 Plates and spin in a microtiter-plate centrifuge for 60 seconds at 200 RCF.
8. Following centrifugation, place the plates on the supplied Akura™ Tilting Stand in a humidified CO_2 incubator at 37°C for 4 days.

Medium Exchange in the Akura™ 96 Spheroid Microplate

1. Pre-warm the **Maintenance Base Medium** and thaw the **Maintenance Medium Supplement** in a water bath set to 37°C.
2. Wipe pre-warmed Maintenance Medium bottle and Maintenance Supplement tube with 70% EtOH and transfer to sterile working area.
3. Pipet the contents of the **Maintenance Medium Supplement** to the **Maintenance Base Medium** (now **Maintenance Medium**) and mix.
4. Place the pipette tip at the ledge of the well.
5. 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.
6. Add 70 µl of fresh **Maintenance Medium** by placing the pipette tip at the ledge. Use a dispensing rate <50 µl/sec.
7. Place the lids on the Akura™ 96 plates and return them to the incubator.



Medium exchange in the Akura™ 96 Plate. Left: Medium removal with the pipette tip placed at the ledge of the well. Right: Medium addition.

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