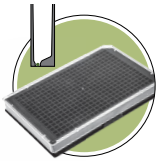


Akura™ 384 ImagePro
Quick Start Guide

Thank you for choosing InSphero's Akura™ 384 ImagePro 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 on shop.insphero.com.

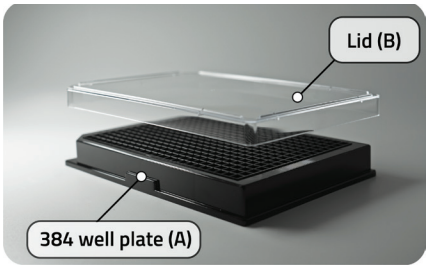


Figure 1. Akura™ 384 Plate components.

Akura™ 384 Plate Components

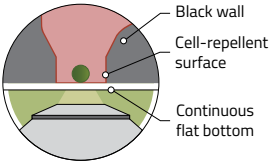
A. Akura™ 384 well plate

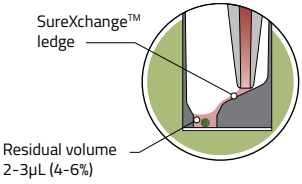
B. Transparent lid

Advantages of the Akura™ 384 ImagePro

1. Convenient scaffold-free formation of 3D cell models via cellular self-assembly in ultra-low attachment (ULA-treated) plates.

2. The continuous, highly transparent, 25µm bottom results in enhanced imaging quality and depth, and the black-walled body eliminates fluorescent crosstalk between wells.

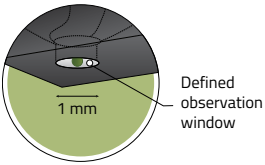




3. SureXchange™ tapered ledge and culture chamber facilitates easy medium exchange and prevents spheroid/organoid loss during long-term spheroid growth and analysis.

4. 1 mm diameter flat bottom observation chamber enables simple spheroid/organoid localization, observation and ROI identification.

5. Akura™ 384 Plate is compatible with state-of-the-art imaging and automated liquid handling systems enabling HTS applications.



Generating 3D Cell Culture Models Like Spheroids or Organoids

Important: *In order to prevent inclusion of air bubbles, is is recommended to pre-wet the wells of the Akura™ 384 Plate. Apply 40µl of cell line medium containing FCS or BSA to each well by placing the tip near to, but not touching, the bottom of the well.*

1. **Cell seeding:** Count the cells and prepare a cell suspension for seeding, using a final volume per well of 50µl. For a long-term growth profiling start with low cell numbers (250 – 500 cells per well). If non-proliferating cells or rapid production of larger spheroids are required, then start with higher numbers (2,500 cells or more per well). You may wish to try several different concentrations to define your optimal range.

Important: *Ensure a homogeneous distribution of the cell suspension by gently pipetting up and down prior to seeding. Also, gently add 50µl of cell suspension (≤ 10 µl/sec) by placing the pipette tips near to, but not touching, the bottom of the wells.*

2. **Sedimentation spin:** It is recommended to briefly centrifuge the plate for 2 minutes at 250 RCF to remove air bubbles.
3. Tilt the plate in the incubator to approximately 30° to improve the maturation process.
4. Incubate the plate in a humidified CO_2 incubator at 37°C. Spheroid maturation typically occurs within 2-5 days of seeding depending on the cell type and culture conditions.

Medium Exchange in the Akura™ 384 ImagePro

1. Place the pipette tip at the ledge of the well (Fig. 2).
2. Remove the medium at low pipetting speed (<30 µl/sec) by aspirating an excess of volume. A minimal volume of ~2-3µl medium will remain in the well.
3. Add 50µl 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™ 384 Plate and place it in a humidified CO_2 incubator at 37°C.

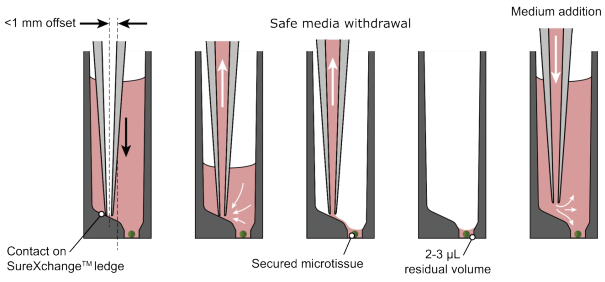


Figure 2: Safe medium exchange in the Akura™ 384 Plate.

For detailed information, please refer to the Akura™ 384 Plate 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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