Frequently Asked Questions Regarding the Akura™ 384 Spheroid Microplate

A protocol for production of spheroids in the Akura™ 384 Plate is provided in the product manual. Below are answers to some frequently asked questions to help get you started.

Q: What is the difference between Akura™ 384 Spheroid Microplate and the Akura™ 384 ImagePro Plate?

A: Both Akura™ 384 Plates have black walls to minimize cross-talk und an unique well design for near-complete medium exchange without spheroid loss.

The Akura™ 384 ImagePro has a flat, ultrathin, 25 µm gas-permeable membrane made of FEP (fluorinated ethylene propylene) to minimizes RI mismatch and it is compatible with high NA objectives.

The Akura™ 384 Spheroid Microplate has a 125 µm thin Polystyrene membrane. The plate is especially suited for high throughput applications, lytic and biochemical assays, and basic confocal imaging endpoints.

If you require a high-resolution, high-content imaging endpoint, we recommend the Akura™ 384 ImagePro Plate.

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

A: Pre-wetting the wells of the Akura™ 384 Plate is recommended prior seeding to prevent inclusion of air-bubbles. For pre-wetting, apply 50 µl of PBS to each well by placing the tips near to, but not touching the bottom of the well.

Centrifuge the Akura™ Plate for 2 minutes at 250 RCF and incubate it in a humidified CO_2 incubator for at least 1 day. Before cell seeding take the Akura™ Plate from the incubator, centrifuge the Akura™ Plate for 2 minutes at 250 RCF. Aspirate the PBS by placing the tip at the ledge of the upper cavity of the well. Aspirate until the PBS is removed from each well. A small amount of PBS (< 2-3 µl) remains in the bottom of the chamber.

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 well of 50 µl). If use of non-proliferating cells or rapid production of larger spheroids are required, start with higher numbers (from 2500+ cells per 50 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™ 384 Plate?

A: To achieve optimal conditions, gently deliver 50 µl (pipetting speed < 10 µl/sec) of cell suspension into each well of the Akura™ 384 Plate by placing the pipette tips far into the wells (avoid touching the well bottom).

Important – To obtain 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™ 384 Plate.

Q: Why do you recommend centrifuging the Akura™ 384 Plate 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 microplate 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™ 384 Plate without disturbing or losing the spheroids?

A: To prevent spheroid loss during the exchange of media, place multi-channel pipette tips at the ledge by slowly sliding down along the inside of the well wall (angled slightly toward the top of the plate) until a subtle resistance can be felt.

Note: Proper aspiration with a multi-channel pipette is possible only row-wise. Carefully and slowly remove the medium by aspirating an excess of volume (> 50 µl). This will lead to an almost complete removal of the medium, with a consistent residual medium volume.

Add 50 µl of pre-warmed medium by placing the pipette tip at the ledge of the plate well and gently dispense at a slow pipetting speed. Never allow the pipette tip to touch the bottom of the well as it consists of a 125 µm thin membrane.

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™ 384 Plate, at least 40-50 µl of medium in each well is recommended. 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 13) to reduce edge effects when performing long-term culture with low-frequency medium exchange. The InSphero Incubox™ is available on shop.insphero.com.

Figure 13: InSphero Incubox™

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

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