Frequently Asked Questions Regarding Akura™ Twin

A detailed experimental protocol for the Akura™ Twin Microplate is provided in the product manual. Below are answers to some frequently asked questions to help get you started.

Q: What are the applications of Akura™ Twin Microplate?

A: The first application we are presenting is a tumor-liver co-culture combined with immune cells. We show how cytokines released by the immune cell – tumor interaction affects the liver. You can develop a broad range of other applications, in which co-culture and organ-organ communication plays an important role for your experimental question. Basically, all spheroid models in health and disease can be used. Please consider our checklist towards successful experiments:

- ✓ Organs models can be produced as scaffold-free spheroids in Akura™ 96 or 384 Microplates, or purchased at InSphero
- ✓ Organ models show a compact morphology for pipet transfer with a diameter of less than 800 µm over the duration of the experiment.
- ✓ Quality control criteria for organ models have been established to assess function prior to co-culturing experiments
- ✓ Common media for co-culturing has been established preserving relevant function of the individual organ model
- ✓ Protocols for immune cell expansion and staining has been established, or Immune cells and respective protocols are ordered at InSphero
- ✓ Common media preserves function of immune cells
- ✓ Read outs for individual organ model and immune cell monitoring have been established

Q: What are the dimensions of the spheroid chamber and the channel between two chambers?

A: The spheroid chamber has a diameter of 1 mm and measures 600 µm in height. The channel is 1.6 mm wide and 90 µm high. Center-to-center distance of the wells is 4.5 mm, which is the standard 384-well pitch. Please consult the Technical Specifications for more details.

Q: How is the flow generated?

A: For perfusion, the plate is placed on the Akura™ Programmable All-in-One Tilter inside the incubator and the plate is tilted back and forth to induce flow between the wells by gravity. The plate is rotated around the long axis (landscape tilting).

Q: Why are some channels at the edge perpendicular to all other channels?

A: These conditions can be used as “no-flow” controls, because the channels lie on the rotational axis and both wells are always on the same height.

Q: Can I aggregate spheroids in the plate?

A: No. The well design does not allow consistent cell aggregation. Further, many organ models need specific production media and protocols, which not compatible with each other and why co-aggregation is not recommended. Spheroids need to be aggregated in another plate - preferable in one of the Akura™ plate family or purchased assay-ready from InSphero. This allows robust production using their specific protocol and QC before you transfer them. Once formed, you transfer them into the Akura Twin assay plate for co-culturing. Transfer can be done manually (for a small number of replicates), semi- or fully automated. There are protocols available for Opentrons OT-2 liquid handler. Please contact us.

Q: What is the material of the Akura™ Microplate?

A: The plate is made of Polystyrene and the bottom membrane of Cyclo-Olefin-Polymer (COP), with a thickness of 188 µm. Cyclo-Olefin Polymer is a highly transparent material (same range as glass) with 92% transparency at wavelengths 400-800 nm, and therefore compatible with high-resolution imaging. The well and microchannel walls have cell-repellent properties to preserve long-term spheroid morphology and function and prevent cell adhesion.

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

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