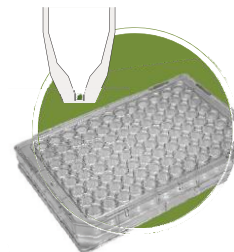


3D InSight™ Microtissues

Quick Start Guide



Instructions for handling 3D InSight™ Liver and Islets Microtissues delivered in Akura™ 96 Spheroid Microplates upon arrival.

1. Please check the box for any damage that may have occurred during transport. If the box is damaged, contact InSphero customer service.
2. Remove the bag containing Akura™ Plates from the shipping box.
3. Remove temperature logger from shipping box. Verify that green "Recording" LED is blinking intermittently. Stop the logging process by pressing the "Start" button for 5 seconds (the logger confirms recording-stop by flashing 10x).

Press the "Start" button again:

- a. "Recording" LED flashes green 2x = no temperature exception detected -> proceed with experiments
- b. "Status" LED flashes red 2x = temperature exception* detected -> please plug the device into a computer USB port and send the saved logging files to: distribution@insphero.com

Accepted temperature zone (SZ) = 14 °C – 40 °C / Higher temperature limit (H1) = 40 °C / Lower temperature limit (L1) = 14 °C

4. Wipe the outside of the bag with 70% ethanol and dry. Carefully open the sealed bag and remove the Akura™ Plate containing the microtissues.
5. **Do not remove the sealing membrane** on the Akura™ Plate as some microtissues may stick to the seal and need to be recovered by centrifugation.
6. **Centrifuge the Akura™ Plate with intact membrane** at 250 rcf for 2 minutes at room temperature (each well contains 70 µl of shipping medium - total weight of plate ~63 g).
7. Place the Akura™ Plate under a microscope to verify each well contains one microtissue. If all microtissues are present, remove the sealing membrane (Figure 1 and 2). If some wells appear to be empty, please consult troubleshooting instructions on the backside of this guide or contact InSphero (support@insphero.com)
8. Perform a medium exchange on the tissues according to the instructions in the Technical Protocol (TP007).
9. Allow the microtissues to rest in incubator before starting the experiments. Resting time varies by tissue type:

For **Liver Microtissues** – Overnight

For **Islet Microtissues** - Overnight

10. Plan to exchange medium every 2-3 days until you are ready to run assays.

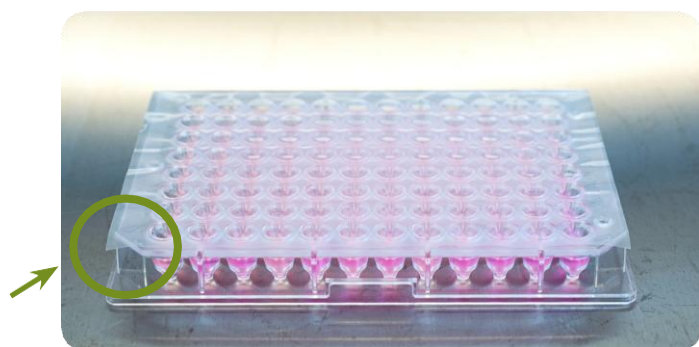


Figure 1: On the lower left side, the membrane overlaps the plate. Start with the lower left corner and remove the seal starting with the first column from left to right, column by column.



Figure 2: Hold the plate firmly with your left hand while applying downward pressure, then remove seal with your right hand until the whole membrane is released.

Troubleshooting Instructions

Under certain circumstances, microtissues can attach to the plate seal or may adhere to the air-liquid interface due to surface tension interactions. If microtissues appear to be missing after step 7 of this Quick Start Guide, please follow the steps below to increase the chances for successful recovery of microtissues.

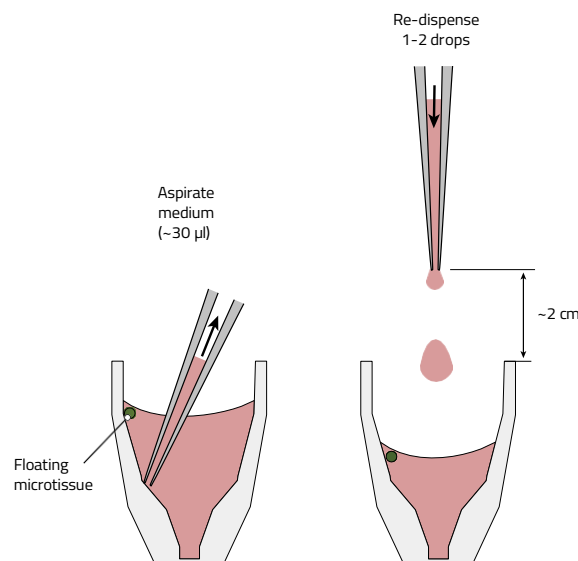


Note: This troubleshooting protocol is not appropriate for all microtissue types.

- Centrifuge the Akura™ 96 Plate with seal intact again in a microtiter plate centrifuge at 250 rcf for two minutes at room temperature.
- Verify the presence of microtissues at the bottom of the wells using an inverse brightfield microscope.

If any microtissues appear to be still missing, repeat steps a. and b. Persistent failure of complete recovery may be related to microtissues sticking to the air-liquid interface. In this case, proceed to steps c-f.

- Place plate under a laminar flow hood, remove lid and gently peel off seal (Figure 1 and 2).
- Remove 30 μ l of medium from the well and re-dispense the same medium from above at a distance of ~2 cm and release 1-2 drops into the well (see illustration below). This will cause any floating microtissue to sink to the bottom of the well. Alternatively, fast dispense medium directly at the well wall on top of the microtissue for proper recovery into the well.



- Verify presence of microtissues at bottom of wells using an inverse brightfield microscope.
- After successful recovery of the microtissues, proceed with step 8 of this Quick Start Guide.

Disclaimer: InSphero guarantees that in all plates shipped all wells destined to be filled contained microtissues when packed for transport. This is confirmed via optical scans of each plate shipped. On rare occasions during transport, a few microtissues may be damaged. InSphero uses a protective packaging system to minimize microtissue loss due to shipping and handling and guarantees that $\geq 95\%$ of microtissues will be available for use upon arrival at the customer site.