Organotypic tumour slice culture (OTSC)

Sample: fresh tumour tissue
Reagents and materials: PBS, low-melting agarose, superglue, acetone, 12 or 24-well plates
Compresstome® settings:

- Slice thickness: 300 µm
- Speed: 2-3
- Oscillation: 3-5

Medium:

- RPMI 1640
- 5 % FBS
- 1 % GlutaMAX™
- 1 % Penicilin-Streptomycin
- 5 µg/mL Insulin
- 1 mM N-acetylcysteine

The final composition of the media depends on the experimental design, treatment conditions, and intended purpose of the study.

A. Establishment of OTSCs using Compresstome®

1. Prepare the medium according to the composition described above.
2. Add 1 mL of medium into a 24-well plate or 1.5 mL into a 12-well plate. If no treatment will be administered, a larger volume of medium can be used, though excessive medium can affect **oxygenation.**
3. Pre-warm the plates containing medium at 37 °C.
4. Prepare the Compresstome® machine. If it is not already inside the sterile hood, place it in the hood, disinfect it thoroughly with ethanol (especially the tub), and expose it to UV light for sterilization. **NOTE: KEEP IN MIND THAT THE TUB AND METAL PART OF THE CYLINDER CAN´T BE WASHED WITH ACETONE!** Other parts, such as the razor holder and the plastic part of the cylinder, where superglue was used, can be cleaned with acetone.
5. Prepare the sample cylinder and razor holder.
6. Attach the razor blade to the holder using superglue.
7. Insert the razor holder with the attached blade into the Compresstome®.
8. Prepare the tissue according to your experimental preference.
9. Apply a small amount of glue onto the plastic part of the cylinder and attach the tissue to it. Allow the glue to dry completely.
10. Prepare 2% low-melting agarose, melt it, and allow it to cool to approximately body temperature.
11. Once the agarose reaches the optimal temperature, embed the tissue by gently applying the agarose in circular motions around the sample. **NOTE: DO NOT POUR HOT AGAROSE ON GLUED TISSUE!**

12. Cool the cylinder using the chilled holder.
13. Set the vibratome conditions (slice thickness, speed and oscillation as required) and insert the cylinder into the tube.
14. Adjust the piston carefully so that the sample is firmly secured and advances smoothly during sectioning.
15. Fill the tub with chilled PBS to a level slightly above the sample but below the upper edge of the tub.
16. Check all settings and begin sectioning. Start with a single section to confirm proper cutting conditions, then continue with continuous slicing. It is recommended to initially cut slightly thicker sections than desired and gradually reduce to the target thickness.
17. Using forceps, carefully collect the slices and transfer them into the prepared wells.
18. Cultivate OTSCs at 37 °C in a humidified atmosphere with 5 % CO_2 for the desired incubation period.

B. Resazurin viability assay

Important: Resazurin is light sensitive and should be protected from light whenever possible.

1. On the final day of the experiment, remove all medium from the wells. Prepare a stock solution of resazurin (10 mg/mL) and dilute it 1:150 in the same medium that was used during the experiment.
2. If using a positive control, select one well at the beginning of the experiment, and before adding resazurin, remove the medium and incubate the slices in 70% ethanol for 1 hour.
3. After the 1-hour incubation at 37 °C and 5% CO_2, remove the ethanol and wash the slices 2–3 times with PBS.
4. Add 1 mL of the diluted resazurin solution into each well of a 24-well plate. The volume may vary depending on the plate format, but all slices must be fully covered by the solution.
5. Prepare a blank control by selecting an empty well or an Eppendorf tube containing only the diluted resazurin solution. Incubate the blank under the same conditions as the treated samples.
6. Incubate the plate for 4–6 hours at 37 °C and 5% CO_2, protected from light.
7. After incubation, transfer 100 µL from each well into a 96-well plate.
8. Measure fluorescence at the appropriate wavelength settings (e.g., Excitation: 545 nm, Emission: 600 nm; Braun et al., 2021) for resazurin/resorufin detection.

NOTE: When performing the resazurin viability assay, a positive control (ethanol-treated well) should always be included to confirm that the assay is functioning correctly.