OCT Cryosections

For the evaluation of certain proteins or other cellular components, routine formaldehyde fixation and paraffin-based histology is not suitable.

In these situations, sections should be embedded in OCT medium and cryosectioned to retain the most sensitive and undisrupted insight into tissue architecture.

Materials

OCT

Dry ice

Pointed forceps

Scalpel

Aluminium foil

-80°C freezer

Protocol

1. Prepare small aluminium foil cups using a small bottle or other round object as a circular mold. Cups should be 1cm diameter, 2cm high
2. Cut the membrane with the 3D epidermal culture carefully out of the plastic insert. Once removed, keep the membranes in a 24well plate containing some medium, until all membranes are cut out and you are ready to proceed.
3. Take one membrane at the time out of the medium, place it onto a clean surface (Petri dish) with the membrane downwards, and cut two parallel strips off opposing sides, leaving the main central strip remaining (approx 5 mm wide) .
4. Add a drop of OCT into an aluminium cup and place it onto a plate of dry ice until the bottom starts to solidify and turn white.
5. With the cup still on the dry ice, place the membrane strip onto the OCT and cover it carefully with some more OCT. The whole tissue should just be covered with OCT. There should no air bubble be trapped, if one is present try to displace it to the edge. It is important to note the orientation of the tissue using a permanent marker.
6. When all of the OCT has hardened (white), label the block (e.g. with a small strip of paper), then store at –80°C.
7. Before cutting place the embedded tissue into the cryo-sectioning machine set to –20°C and leave it there for about 15 minutes to adjust to this temperature.
8. The block must be mounted onto the specimen holder in the correct orientation to obtain the desired cuts. Trim the block as necessary to allow the desired orientation.
9. Cut sections at 4 um. For immune-histology, we recommend to mount the slides on silane coated slides.

In case of further questions, please email out scientists directly: scientist@cellntec.com