Histological Processing and Staining

3D Epidermal models established using our 3D starter kits can be processed using routine histological procedures, and stained with H&E to visualize the structure of the model. Specific antigens may also be visualized by immunohistology. We recommend the following procedures, most of which may also be seen in the 3D training video available on www.cellntec.com

Materials

4% PFA

Pointed forceps and scalpel

Embedding casettes with corresponding foam biopsy pads

Reagents and equipment for histological processing, paraffin embedding, and sectioning

Reagents for H&E staining, and IF staining if desired

Protocol

1. Fixation of cells on insert:

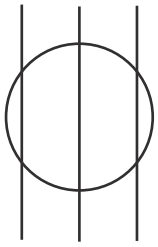
- Place insert in spare well of a culture plate
- Carefully fill the whole insert and the well with 4% PFA (in PBS) and incubate over night at 4°C

2. Cutting the membranes out of the inserts:

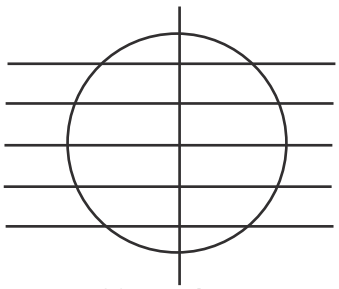
- Use a new scalpel blade.
- Carefully remove the membrane from the insert by cutting around one half of the membrane from the outside of the insert, then leave a small bridge and cut the remaining half. When reaching the starting point carefully place the membrane on a drop of liquid (PFA) on a smooth surface (petri dish) and carefully cut the remaining bridge from inside the insert (if the bridge is very small the membrane can be removed by simply holding the membrane while tearing the insert away).

3. Preparation for paraffin embedding:

- Cut the membrane according the following scheme (discard smallest slivers)



12 mm Insert



30 mm Insert

- Place the membrane pieces between 4% PFA-soaked biopsy foam pads and place into embedding cassettes (these pads are important to reduce forces on the cell layer during processing).

4. Processing and paraffin embedding:

- We use the following processing program (on Medite Tissue Processor TPC 15):

0h:30min	70% EtOH	37°C
1:30	80% EtOH	37°C
1:30	80% EtOH	37°C
1:30	96% EtOH	37°C
1:30	96% EtOH	37°C
2:00	100% EtOH	37°C
2:00	100% EtOH	37°C
1:30	Xylol	37°C
2:00	Xylol	37°C
1:30	Paraffin	62°C
1:30	Paraffin	62°C

-Once in paraffin, the sections are ready to be embedded in blocks.

-Half fill a base mold (preferentially stainless steel) with paraffin and place it briefly put on a cold plate to slightly solidify the paraffin at the bottom of the mould.

-Quickly score a straight and narrow channel into the solidified paraffin using the tip of the heated forceps, then remove a membrane section from the cassette and place it in an upright position into the line (hold one edge of the membrane with the heated forceps until the membrane stands on its own). Experienced users may be able to embed two sections in a single block, but this requires both sections to be emdedded are at exactly the same height.

-Carefully add some more paraffin and embed the cassette base into the paraffin block holder.

5. Sectioning:

-Cut 4 micrometer longitudinal sections with a microtome, then mount the sections using a Mounting Bath at 50°C on “Superfrost Plus“ slides (Menzel Gläser J1800AMNZ), 2 sections per slide. For immunohistology, silan-coated slides are recommended for optimal adhesion.

Dry the slides in an oven for 30-45 minutes at 60°C. (For immunohistology dry slides over night at 37°C)

6. H&E Staining:

-H&E staining is done according to the following program (MediteTissue stainer TST 40), with 10-20 seconds of dripping between each step to reduce cross contamination:

3x 30sec	Xylol
2x 30sec	100% EtOH
2x 30sec	96% EtOH
30sec	80% EtOH
30sec	H2O
2x 4min	Hematoxylin Ehrlich's
30sec	H2O
30sec	HCl-EtOH
5min	H2O
3min	Eosin
2x 30sec	96% EtOH
4x 30sec	100% EtOH
30sec	Xylol

-Mount coverslip with Pertex Mounting Medium (with Medite/Meisei Promounter RCM 2000)

7. Immunohistology:

Where desired, specific antigens of interest may be visualised using immunohistology.

1. Deparaffinze slides by processing them according to the following procedure
2. Antibody retrieval (here shown the microwave boiling method, other methods may be preferred, depending on the antigen of interest, please consult the antibody datasheet for more information): Boil tissue sections in a Joplin jar with 10mM citrate buffer pH6.0 2x5min (700 watt microwave).

Hint: To avoid evaporation of the buffer and drying of the slides, we place the Joplin jar into a plastic beaker with holes in the lid, the beaker contained water to the half height of the jars. There is space for 3 Jars in our beaker, non-used jars are just filled with water.

3. Remove the jar from the beaker and let cool to RT for 30min.
4. Wash with PBS+ 2x5min.
5. Surround tissue with DAKO Pen (or other suitable silicon pen).
6. Block unspecific binding with 2% BSA in PBS+ for 30min.
7. Wash with PBS+ 3x5min.
8. incubate with primary antibody for at least 2h in a humid chamber (the tissue should be well covered with Antibody solution).
9. Wash with PBS+ for 3x5min.
10. Incubate with a suitable secondary antibody (normally done for 1h in the dark -Fluorochromes).
11. Wash with PBS+ for 3x5min.

11a. Optional: Nuclear staining: 1:5000 dilution of 1mg/ml Hoechst 33342 in PBS+ for 30sec. (Easiest done by sliding the slide into a 50ml tube containing the Hoechst).

11b. Wash with PBS+ for 3x5min.

12. Do NOT air dry.

13. Immediately mount with mounting medium (e.g. DAKO fluorescent mounting medium S3023) using cover glasses (eg. Marienfeld 24x50mm No.1), add enough mounting medium to avoid air bubbles:

Hint: 5-6 drops distributed over the whole slide are enough, cover with the glass by tilting it from one side, use a pointed tool (eg. forceps) to streak out bubbles.

14. Let the slides dry at 4°C in the dark over night before examination with fluorescent microscope.

PBS+: PBS containing 0.9 mM Ca^{2+} and 0.5 mM Mg^{2+}.

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