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COMPETENCE IN SKIN PHYSIOLOGY

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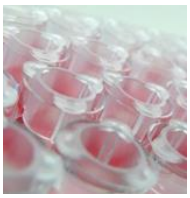
Phenion® FT Skin Model
Histological processing
♦ Frozen sections



Objective

This Standard Operation Procedure is recommended to freeze Phenion® FT Skin Models in order to prepare frozen sections. Frozen sections are especially suited for immunohistochemistry and immuno-fluorescence methods because the antigenic properties of e.g. proteins in the tissue are preserved best after freezing. Antigen retrieval protocols are not needed.

Remark: The process described in this SOP has been adapted for a Thermo Fisher NX70 Microtome. When using other microtome brands the protocol must be adjusted to the requirements of the respective gadget.

Materials

Items	Company	Order-No.
Cryomicrotome	Thermo Fisher	Type NX70
Scalpel with curved blades	Braun (Tuttlingen, Germany)	5518059
Tissue freezing medium	Leica	14020108926
Grooved specimen discs	Thermo Fisher	
Glass slides (Superfrost Plus)	VWR	631-0108
Freezer (-20°)	---	---

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Procedure

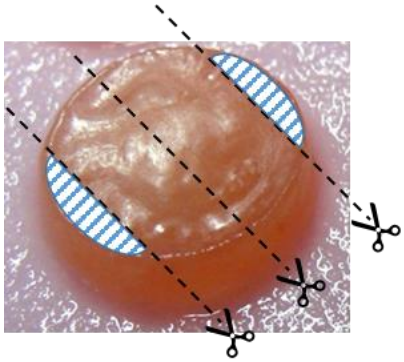
Dissection of skin equivalents

1. Transfer the skin model with tweezers from the air-liquid interface culture vessel onto the lid of a polystyrene petri dish (Ø 10 cm).

2. Carefully cut the tissue into nearly equal stripes using a scalpel with a curved blade. Gently press the scalpel blade onto the tissue surface, then start moving the blade downwards.

3. First bisect the skin model into two nearly identical halves. Then cut the tissue halves again parallel to the first section plane. Discard the smaller curved parts of the tissue (the highlighted parts in the illustration).

4. The 2 remaining tissue stripes are suited to be processed for cryosectioning.



Embedding

1. Place a grooved aluminum specimen disc in the -55°C freezing bar located in the interior space of the cryomicrotome. Cover the disc surface with a thin layer of tissue freezing medium.

2. When the tissue freezing medium has been frozen completely (it has become opaque) place the skin model stripes/pieces side by side with the larger cutting edge facing upwards and leaving a small space between each other (the tissue stripes must not touch each other). Up to 3 tissue stripes can be placed on a single specimen holder.

3. Add tissue freezing medium stepwise around and in the spaces between the tissue stripes until the specimens are completely covered. Make sure to add fresh medium in time before the previous portion has been frozen completely.

4. Repeat the procedure until the specimen is completely embedded in frozen medium. Wait some more minutes to ensure that the inner parts of the tissue are completely frozen, too.

Tissue sectioning in the cryomicrotome

1. Before the specimens can be sectioned properly, the sample temperature must be adjusted to -20°C. Therefore remove the specimen holder with the frozen skin tissue samples from the freezing bar and place it in the object head. The skin model stripes must be aligned perpendicular to the microtome blade (at a 90° angle). This orientation is a prerequisite for obtaining homogeneous sections of the complete tissue. Make sure that the disc is clamped tightly in the object head. After a few minutes the temperature in the frozen tissue is equilibrated, and the cutting process can be started.

2. Follow the instructions of your cryomicrotome to prepare a sufficient number of tissue sections.

3. Cut the skin model tissue into sections of 7 µm thickness and transfer them to Superfrost Plus slides.

4. Remove the glass slides with the sections from the cryomicrotome. Let them dry for 15 minutes at room temperature before proceeeding with tissue staining, e.g. with hematoxylin and eosin (H&E) staining or for immunostaining.

5. The tissue sections can be stored at -20° until further use.

6. For storage of specimen blocks seal it e.g. in aluminum foil to prevent exsiccation. Store at a minimum of -20 °C.

Attention: Keep the storing time for the specimen blocks in the cold as short as possible in order to guarantee best cutting results. Prolonged storage can lead to exsiccation and hardening of the freezing medium which eventually might prevent proper cutting.