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phenion
COMPETENCE IN SKIN PHYSIOLOGY
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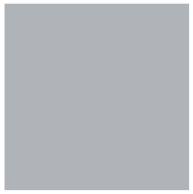
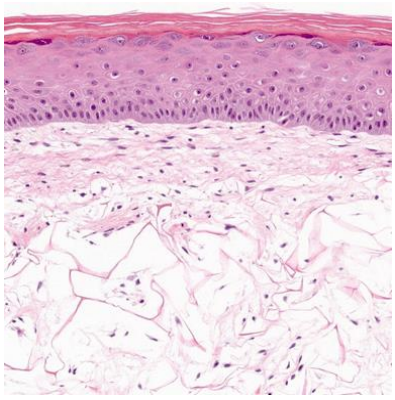
Phenion® FT Skin Model
Histological processing
♦ Paraffin sections





Objective

This Standard Operation Procedure is recommended to fix and embed Phenion® FT Skin Models in order to prepare paraffin sections. Paraffin sections, after staining e.g. with hematoxylin/eosin, are especially suited to demonstrate tissue architecture with high resolution. It can also be used for immunohistochemistry and immunofluorescence methods, however, antigen retrieval protocols might be needed to generate a specific signal.

Materials

Items	Company	Order-No.
Scalpel	---	---
Curved tweezers	---	---
Embedding cassettes	e.g. Thermo Scientific	C-250-AQ
Petri dishes with lid	e.g. Greiner Bioone	---
Embedding molds	VWR	720-0824
Fine paintbrush	Bernshaus Düsseldorf	1570
Microscope slides Superfrost Plus	VWR	631-0108
Coverslips 24x50mm	Carl Roth	1871
Slide autostainer	Thermo Scientific	Gemini AS
Automated tissue processor	Sakura	Tissue-Tek Vip5 Jr.
Formaldehyde solution 4% buffered, e.g. Roti Histofix	Roth	P087.3
Ethanol (50 - 100%)	e.g. Sigma	---
Isopropanol	e.g. Sigma	---
Xylene	e.g. Sigma	---
Paraffin Paraplast Plus	Sigma	P3683
Hematoxylin	Richard-Allan-Scientific	7211

Tap water	---	---
Ammonium hydroxide solution 25%	e.g. Sigma	221228-1L-M
Eosin	Shandon	6766007
Mounting medium	Thermo Scientific	8312-4
Embedding station	Leica	EG1140H
Plate freezer	Leica	Histo Core Arcadia C
Microtome	Leica	RM2145
Microtomic blades	Thermo Scientific	3052835
Water bath	Kunz Instruments	HIS-2

Procedure

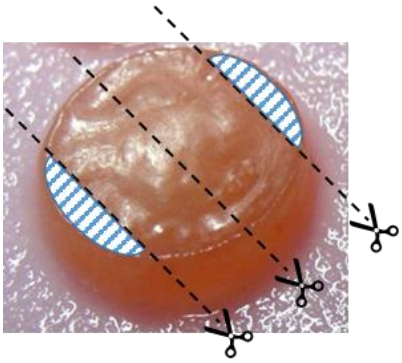
Fixation and embedding of the skin models

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.

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



5. Sign the the embedding cassette for proper sample identification. Use a pencil for signing the cassette because it resists organic solvents best. Position the tissue pieces carefully in the embedding cassette and close the lid.

6. Then immerse the cassette in a 4% buffered formaldehyde solution. Use stained bottles for storing in order to prevent light exposure.

7. Place the bottle containing the cassettes at least 24 hours in the refrigerator for optimal fixation. The tissues can be kept for a longer time in the formaldehyde solution, but be aware that antigen retrieval might suffer with increasing fixation time.

8. Remove the embedding cassette with the tissue stripes from the formaldehyde solution with tweezers and rinse it in tap water for 15 minutes before starting the dehydration process.

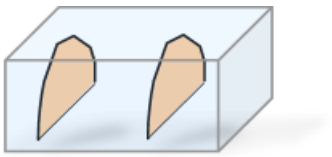
9. The fixed skin models must be dehydrated in order to completely remove water and exchange it against liquid paraffin. The following protocol is intended for use in an automatic tissue processor. If no tissue processor is available, the protocol might be adapted accordingly.

-	Ethanol 50%	1 hour	35°C
-	Ethanol 70%	1 hour	35°C
-	Ethanol 70%	1.5 hours	35°C
-	Ethanol 90%	1 hour	35°C
-	Ethanol 95%	1 hour	35°C
-	Ethanol 95%	1 hour	35°C
-	Isopropanol	1.5 hours	35°C
-	Isopropanol	1.5 hours	35°C
-	Xylene	1.5 hours	35°C
-	Xylene	1.5 hours	35°C
-	Paraffin	1 hour	60°C
-	Paraffin	1 hour	60°C
-	Paraffin	1 hour	60°C

10. Open the lid of the embedding cassette, carefully remove the still warm skin model stripes and place them on the prewarmed working area of the embedding station.

11. Fill a prewarmed embedding mold with liquid paraffin (60°C).

12. Then place the specimens in the paraffin- filled embedding mold as illustrated below:



13. Orient them with the cutting edge downwards on the bottom of the embedding mold.

14. When the specimens are correctly positioned, place the embedding cassette on top of the filled embedding mold.

15. Add enough paraffin into the embedding cassette until it is completely filled.

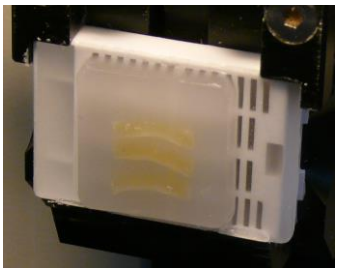
16. Carefully place the molds on the freezing plate of the embedding station. Once the paraffin is completely solid, the blocks containing the tissues can be removed from the mold and stored at room temperature.

Cutting of the tissue

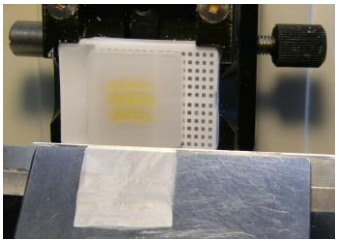
1. Place the embedded samples on a freezer plate (-6°C) prior to cutting for at least 1 hour. The paraffin temperature is a critical parameter to carry out good sections.

2. Prepare a water bath, filled with distilled water at 40-42°C.

3. Place the samples in the microtome. The skin samples must be aligned in parallel to the microtome blade and with the epidermis oriented downwards. This orientation is a prerequisite for obtaining homogeneous sections of the complete tissue.



4. Trim the blocks to remove redundant paraffin and to obtain a plain cutting surface. Discard the first tissue slices to get rid of damaged parts of the tissue caused by scalpel use.



5. Prepare sections with a thickness between 5 and 8 µm.

6. Remove the paraffin sections from the blade with the help of a wet paintbrush and place them carefully onto the surface of the prewarmed water in the water bath. Take care that the sections do not fold, collapse or coil while transferring them onto the water surface.

7. After the sections have become expanded to their final size transfer them on a glass slide. This can be achieved best by lowering one side of a glass slide underneath the expanded section and then lifting it slowly with the sections adhering to the glass surface. Place the slides with the sections facing upwards on a prewarmed heating plate (~40°C) so that residual water can slowly evaporate.

8. Sections must be perfectly dry before staining. Using a drying cabinet (40°C, without humidity) over night for best results.

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Staining of skin model sections

1. A large range of staining options are available which should be chosen according to the user's goals.

   For a quick and clear histological analysis of the skin models we recommend to stain the sections with hematoxylin & eosin (H&E). The H&E staining protocol listed below is intended to be used in an automatic slide autostainer and might be adapted if other equipment is used.

- Xylene	110 sec.
- Ethanol abs.	20 sec.
- Ethanol 50%	20 sec.
- Hematoxylin	230 sec.
- Tap water	20 sec.
- Ethanol 70%	20 sec.
- Ammonium hydroxide ethanol* (1.5%, 80%)	20 sec.
- Ethanol 95%	20 sec.
- Eosin	20 sec.
- Ethanol abs	20 sec.
- Ethanol abs	20 sec.
- Xylene	20 sec.

   *Prepare an 80% ethanol/1.5% ammonium hydroxide solution by adding aequos ammonium hydroxide solution (~25-30%) to 100% ethanol.

2. Maintain the sections in the last cuvette containing xylene until coverslipping with an appropriate mounting medium.

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Typical H&E section of a paraffin embedded Phenion® FT Skin Model: