Precautions

Phenion® FT Skin Models or any of the accompanying parts of the kit shall NOT BE USED FOR OTHER THAN RESEARCH PURPOSES. DIAGNOSTIC OR THERAPEUTIC USE OF KIT CONTENT IS NOT PERMITTED.

Phenion® FT Skin Models contain components that are of human origin and no known test procedures can ensure the total absence of infectious agents. All cells used were tested and found to be negative for Hepatitis B, Hepatitis C, HIV-1 and mycoplasma prior to Skin Model production. Please refer to respective regulatory and scientific guidelines for handling of biological material. Handling of the products must strictly follow latest state of the art safety precautions.
Phenion® FT Skin Model Kit:

A minimal order quantity for one Phenion® FT Skin Model Kit contains six full thickness skin tissues. Respective kits with higher quantities are available upon request according to customer needs. The Phenion® FT Skin Model Kit contains special materials required for the manipulation and cultivation of the tissues. The material provided with the kit enables individual tissue cultivation in six separated vessels or the joint cultivation (e.g. 2 x 3 or 1 x 6) of the skin models. Please order sufficient ALI-Medium (ready-to-use) separately, in 250 ml aliquots (Calculate 35 ml ALI-Medium for a medium exchange per 6 Skin Models every 2nd day).

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Note: Sterile pipets and forceps are not provided with the kit.

Figure 1: Content of the Phenion® FT Skin Model kit
General Instructions

Handle Phenion® FT Skin Models under sterile conditions only. It is recommended to use for the manipulation and cultivation, a laminar flow hood and an incubator for eukaryotic cell cultures (37°C, 5% CO₂, at saturated humidity.

Upon arrival, remove the tissues immediately from the semi-solid transport medium according to the guidelines below. Please follow the instructions, for the cultivation of the tissue in the Air-Liquid-Interface (ALI) up to 9 days. Please note, a longer cultivation might be possible, but will ultimately lead to a Stratum corneum with aspects of hyperkeratosis.

Precautions / recommendations:

- Perform all steps under sterile conditions (respective lab trainings might be recommended).
- It is recommend to use exclusively the accessories (e.g. ALI-Medium, filter and filter spacers, etc.), provided with the kit in order to ensure best cultivation conditions.

A) Culture of individual skin models separately
(For culturing several tissues in one group go directly to B)

1. Place three small petri dishes in one large petri dish and insert one filter spacer into each small petri dish ensuring correct positioning of filter spacer with pin orientation upwards! (See figure 2.)

Figure 2: Placement of the small petri dishes in the larger ones followed by loading with filter spacer.
2. Add 5 ml of 37 °C pre-warmed ALI-Medium into each small petri dish. The medium level shall reach the upper tip of the filter spacer pins (fig. 3 a).

3. Carefully place small sterile filter papers on top of the spacer units (fig. 3 b) to enable wetting of the filter with ALI-Medium.

**Caution:** After filters are soaked, adjust level of ALI-Medium to ensure intense contact between the liquid phase and filter paper. The ALI-Medium and filter surface shall be on the same level, while the filter must not be over-flooded (fig. 3 c).

*Figure 3:*
*a.* filling of ALI-Medium into petri dish. *b.* placing of sterile filter paper. *c.* final preparation. Please note that the medium level is a critical parameter.
B) Culture of up to six skin models in one group

1. If the Skin Models handled group wise, four sterile filter spacer can be snapped together (fig. 4 c). Place filter spacer in a large petri dish and ensure correct positioning, with **pin orientation upwards!** (See figure 4). Disconnected spacer can be placed in a square arrangement too.

![Figure 4: a., b. The filter spacer inserted separately or c. optionally snapped together](image)

2. Add approx. 35 ml of 37 °C pre-warmed ALI-Medium into the large petri dish. The medium level shall reach the upper tip of the filter spacer pins (fig. 5).

![Figure 5: Square arrangement of filter spacers offers proper contact face for filter paper](image)
3. Carefully place large sterile filter papers on top of the spacer units (fig. 6a) to enable wetting of the filter with ALI-Medium.

**Caution:** After filters are soaked, adjust level of ALI-Medium to ensure intense contact between the liquid phase and the filter paper. The ALI-Medium and filter surface shall be on the same level, while the filter must not be over-flooded. (fig. 6 b).

![Figure 6: Please note that the medium height is a critical parameter.](image)

C) Set-up of Air Liquid Interphase culture system

1. Carefully open the protective cover and sealing of the 24-well plate containing the skin models. Remove the shipping protectors with forceps, granting free access to the full thickness skin models (fig. 7).

![Figure 7: Remove the transport protectors, preventing skin models from damaging during transportation.](image)
2. Very carefully grab the Phenion® FT Skin Models using sterile forceps (see figure 8 a) and transfer them on top of the filter papers. Make sure to avoid any air-bubbles between the tissue and the filter paper. Place one skin model per small petri dish (fig. 8 b) or up to six skin models for the large petri dish (fig. 8 c).

![Figure 8: a. removing the tissue from the transport vessel using sterile forceps. b., c. placing the tissues on top of the pre-soaked filter paper for subsequent culture at the air liquid interphase.](image)

3. After transferring the tissues, check that the filter papers are completely soaked with ALI-Medium. Avoid any air bubbles beneath the filter papers and the tissues. If needed, do not hesitate to add a little of ALI-Medium, ensuring that the basis of the Phenion® FT Skin Models on the filter paper is slight surrounded by the liquid.

4. After the final check, cover the tissue culture systems with the lid of the large petri dish (fig. 9 a, b; do not use the lid from small petri dishes) and transfer them to the incubator for eukaryotic cell cultures.
5. For further cultivation (up to nine days) of the Phenion® FT Skin Models, ALI-Medium shall be changed every second day. For standard cultivations, there is no need for ALI-Medium changes at weekends.

Notice:

Apply test substances (repetitively) into the ALI-Medium to mimic a systemic availability. Alternatively, apply substances or also complex formulations (repetitively) onto the surface of the tissue to mimic topical exposure.

In order to secure a skin barrier function, proper epidermal differentiation processes shall not be impaired. In case liquid droplets (e.g. of ALI-Medium) are on the tissue surface, removal with a sterile cotton swab is recommended.