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Phenion® FT INSERT Skin Model Instructions for Use





Precautions

Phenion[®] FT Skin Models or any of the accompanying parts shall NOT BE USED FOR OTHER THAN RESEARCH PURPOSES. DIAGNOSTIC OR THERAPEUTIC USE 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/-2 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.

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Phenion® FT INSERT Skin Model:

Upon request we offer needed number of inserts, complemented by consumables for successful cultivation of the tissues.

Content of the product shipment

- Ordered number of Full-Thickness Skin Models in Inserts (surrounded by transport agar in a 12 well plate)
- Respective number of 12 well cell culture plates for further cultivation of the skin models in your lab. Switch to supply of 6 well cell culture plates can be conducted on demand.

Note: Please order sufficient ALI medium (product ID ALI CM-250 or medium subtypes w/o phenol red, w/o antibiotics or w/o hydrocortisone) separately. Sterile pipets and forceps are not provided with the kit.



Figure 1: Typical content of the Phenion® FT INSERT Skin Model shipment (Skin model inserts, cell culture medium, 6- or 12-well plate).

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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 nine days. Please note, a longer cultivation might be possible, but will ultimately lead to accumulation of *Stratum corneum* layers with aspects of hyperkeratosis.

Precautions / recommendations:

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

A) Skin models on arrival



Figure 2: Skin models in transport agar plate at arrival.

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- B) Set-up of Air Liquid Interphase cultivation system

 Standard 12-well plate (included with the package content)
- Upon arrival add 0.8 ml of 37 °C pre-warmed ALI medium into each well of provided 12-well plate dependent on number of delivered skin model inserts (fig. 3). Place the inserts in the middle of each well so they are standing on their spacer feet (fig. 4b). Exclude air-bubbles beneath the insert membrane. Medium change is recommended every day.



Figure 3: Transfer of the skin models into 12-well plate.

 The inserts can also be cultivated hanging in the 12-well plate with a larger medium volume. For this, it is recommended to use 2.2 ml of ALI medium per well. During further cultivation with hanging insert position medium change is necessary only every second day.

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3. After the final check, cover the tissue culture systems with the lid and transfer them to the incubator for eukaryotic cell cultures.

C) Alternative set-up of Air Liquid Interphase culture system (6-well plate)

For maintenance of the skin model inserts you might also use a 6-well cell culture plate. Articles for this culture format are not included with the shipment routinely. Please feel free to ask for switching of the cell culture plates from 12-well to 6-well before delivery. Prepare for each skin model insert one well with 2.0 ml of 37 °C pre-warmed ALI medium. Place the inserts in the middle of each well standing on the little spacer feet at their bottom.

You might also hang the inserts into the well, then you will need 5 ml ALI medium per well. In both positions change culture medium every second day.

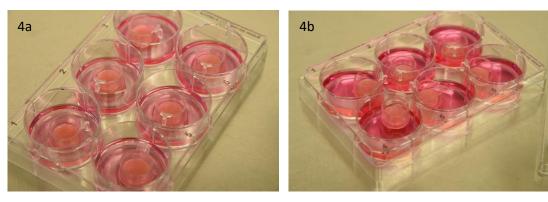


Figure 4:

Possible culture positions (shown in 6-well plate as an example): Standing on spacer feet (fig. 4a) or hanging with hook (fig. 4b).

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6- well culture plate		12-well culture plate	
standing insert	hanging insert	standing insert	hanging insert
2 ml	5 ml	0.8 ml	2.2 ml
Medium change every second day		Medium change	Medium change
		every day	every second day

Overview table:

Possible culture positions of Phenion® FT INSERT Skin Models. Displayed are needed volumes of culture medium and recommended change intervals.

Optional insert feature: The hook for hanging the insert lifted from the bottom might be broken and removed easily with two forceps. This could be useful if the insert needs to be tight fitted in any device for further investigations or treatments.

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 present on the tissue surface, removal with a sterile cotton swab is recommended.