Precautions

Phenion® OS-REp 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® OS-REp 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 OS-Rep 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® OS-REp Model Kit:

Phenion® OS-REp Models can be ordered at any number. The Phenion® OS-REp Models are shipped together with materials required to place the tissues back into culture after arrival at your laboratory. It enables either individual tissue culture in separated vessels or the joint culture of the epidermal models in petri dishes. Please order sufficient OS-REp ALI Medium (ready-to-use) separately, available in 125 ml, 250 ml, and 500ml units. The medium volume required for different culture vessels and tissue numbers is listed in table 1.

<table>
<thead>
<tr>
<th>Content for</th>
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<tbody>
<tr>
<td>6  OS-REp Models (inserts placed on transport agar in a 24-well plate)</td>
</tr>
<tr>
<td>1  sterile 6 well plate</td>
</tr>
<tr>
<td>1  sterile medium-sized petri dish (100/20 mm)</td>
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<tr>
<td>1  sterile large petri dish (145/20 mm)</td>
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**Note:** Sterile pipets and forceps are not provided with the kit.
General Instructions

Handle Phenion® OS-REp Models under sterile conditions only. It is recommended to use a laminar flow clean bench for working with the tissues and an incubator for eukaryotic cell cultures (37°C, 5% CO₂, at saturated humidity) for keeping them in culture.

Upon arrival, remove the tissues immediately from the semi-solid transport medium according to the guidelines below. Please follow the instructions for culturing the tissues at the Air-Liquid-Interface (ALI) for up to 7 days. Please note, that a longer cultivation might be possible, but will ultimately lead to a Stratum corneum with aspects of hyperkeratosis.

Culture of OS-REp models after arrival

1. After arrival at your lab we recommend to culture the epidermal models for 24h (or over night) with the OS-REp ALI medium before starting your experiments. This procedure will increase the overall tissue fitness after shipment.

2. The OS-Rep models can be cultured in sterile plastic dishes of different size: either individually in the wells of a 6 well plate or in in groups in petri dishes.

3. Depending on the chosen culture vessel, the medium volume must be adapted accordingly (Table 1). The indicated volumina are sufficient to support OS-Rep culture over 3 days, which is the time intervall of a weekend (from Friday to Monday).

<table>
<thead>
<tr>
<th>culture vessel</th>
<th># OS-REp models</th>
<th>medium volume</th>
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</thead>
<tbody>
<tr>
<td>6-well plate</td>
<td>1 insert</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Petri dish, ø 10 cm</td>
<td>4 inserts</td>
<td>9 ml</td>
</tr>
<tr>
<td>Petri dish, ø 14.5 cm</td>
<td>10 inserts</td>
<td>23 ml</td>
</tr>
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</table>

Table 1: Medium volumina and OS-REp numbers for different culture vessels
4. Upon arrival fill the respective volume of OS-Rep ALI Medium in each well of the 6-well plate or in the petri dish. The medium has to be warmed up at 37°C prior to use.

5. Remove the plastic foil and the Parafilm® sealing and open the 24 well plate containing the OS-REp models.

6. Remove the OS-REp models with a sterile pair of tweezers and transfer them into the medium-filled wells of the 6 well plate or the petri dish. Carefully remove the filter discs underneath the insert membrane and all remnants of the transport agar from the insert walls.

Fig. 1: The insert containing the OS-REp model is placed into the well of a 6 well plate, filled with prewarmed OS-Rep ALI medium, using sterile tweezers.

Attention: After placing the inserts into the culture vessels make sure that no air bubble has been trapped beneath the insert membrane. If you see a bubble, carefully lift the insert with sterile tweezers and place it once again in the medium. This can be done most successfully if the insert is lowered inclined into the fluid.

Trapped bubbles will lead to malnutrition and subsequent death of the epidermal tissue.
7. Close the lid of the 6-well plate or the petri dish and transfer the models to the incubator. The Phenion OS-REp models must be incubated at 37°C, 5% CO₂ and saturated humidity.

8. The OS-Rep ALI medium must be changed the next day and then every other day (3 times a week, preferably at Monday, Wednesday and Friday) unless the frequency of medium changes must be adapted to the design of your experiments.

Attention:

Immersion into the culture medium or rinsing the surface of the skin models with medium can interfere with the differentiation process and thus can damage the upper layers of the Phenion epidermal model, also impairing the barrier function. In the case that liquid droplets (e.g. of OS-REp ALI Medium) are on the tissue surface, removal with a sterile cotton swab is recommended.
Further reading:

The details of the OS-REp model production process, as well as basic properties and the results of an extensive validation study according to OECD TG 439: “in vitro skin irritation testing” are published in 2 peer-reviewed papers:


The papers also contain the detailed protocol for running an in vitro skin irritation assay for chemicals with the OS-Rep model.