3D Tissue models

*In vitro* skin irritation test - EpiDerm™
Introduction

Three-dimensional (3D) reconstructed tissues are composed of human cells and mimic the architecture of human tissues. They can be used for regulatory safety testing, product development and basic research applications throughout the cosmetic, chemical, pharmaceutical, medical device and household product industries.

Metabolically active tissue models representing different organ tissues with dozens of applications are available along with protocols for a diverse range of assays. Epidermal skin models, for example, can be used in a variety of applications, including corrosion, irritation, sensitization, percutaneous absorption, phototoxicity and genotoxicity endpoints. Lung, intestinal, oral, corneal, vaginal, and other models are also available.

3D tissue models are used in several internationally accepted OECD test guidelines to assess the safety of substances for regulatory purposes, including skin corrosion (guideline 431), skin irritation (guideline 439) and eye irritation (guideline 492).

The skin irritation test detects possible skin damaging effects of medical devices, cosmetics, textiles and other products. Skin irritation testing is performed using animal experiments (in vivo testing). A growing number of these in vivo tests have to be replaced by in vitro tests (animal-free testing) because of legal requirements of animal welfare. In the case of cosmetics all of these in vivo tests have to be replaced by in vitro test systems. These new in vitro test systems use epidermal 3D-skin cell culture models composed of re-cultivated human skin.

Envigo’s in vitro skin irritation testing service uses MatTek’s EpiDerm™ 3D human skin tissue model. This model is considered by OECD as Validated & Accepted alternative method for replacing the Draize Skin Irritation Test. The protocol follows the OECD test guideline 439 and supported by ISO 10993-10, REACH (EU Regulation). Test procedure can be performed under GLP or non-GLP conditions.

EpiDerm™ consists of normal human-derived epidermal keratinocytes, which have been cultured to form a multilayered highly differentiated model of the human epidermis. It consists of organized basal, spinous and granular layers, and a multilayered stratum corneum containing intercellular lamellar lipid layers arranged in patterns analogous to those found in vivo.

Method

The EpiDerm™ tissues are cultured on specially prepared cell culture Millicell™ inserts and supplied as kit.

The test consists of a topical exposure of the neat test chemical to the reconstructed human epidermis (RhE) model for 60 minutes. Negative and Positive Controls are run in parallel. Preferably, three tissues are used per test chemical and controls.

Following exposure, tissues are thoroughly rinsed, blotted to remove the test substances, and transferred to fresh medium. Cells are incubated for additional 42 hours.

Cell viability is measured by dehydrogenase conversion of MTT [(3-4,5-dimethyl thiazole 2-yl) 2,5-diphenyltetrazoliumbromide], present in cell mitochondria, into a blue formazan salt that is quantitatively measured after extraction from tissues. After MTT incubation, the blue formazan salt formed by cellular mitochondria is extracted with isopropanol and the optical density of the extracted formazan is determined using a spectrophotometer at 570 nm.

Cytokine release (optional)

Following 24 hours of incubation period with test materials, the medium is collected for analysis of cytokines.
Evaluation of results

For each Test Item, Positive or Negative Control, the mean relative viability of three individual tissues is calculated and used for classification according to the following prediction model:

According to the EU and GHS classification (R38/ Category 2 or no label), an irritant is predicted if the mean relative tissue viability of three individual tissues exposed to the test substance is reduced below 50% of the mean viability of the negative controls.

<table>
<thead>
<tr>
<th>In vitro result</th>
<th>In vivo prediction</th>
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<tbody>
<tr>
<td>Mean tissue viability ≤ 50%</td>
<td>Irritant (I), (R38 or GHS category 2)</td>
</tr>
<tr>
<td>Mean tissue viability &gt; 50%</td>
<td>Non-irritant (NI)</td>
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Representative Results:

Assessment of the skin irritation potential Test Item X using the reconstructed human epidermis (RhE) model EpiDerm, was evaluated at 3 doses (100, 50 and 25µg/ml) and calculated relatively to the Negative Control mean viabilities. The results show that the all Test Item doses mean viability were > 50% thus interpreted as non-irritant.
Negative control:

Arrow indicates normal epidermal cells

Positive control:

Arrow indicates necrosis of epidermal cells. The cells appear with eosinophilic cytoplasm and no normally present basophilic chromatin in nuclei

Test Item X (100µg/ml):

Arrow indicates normal epidermal cells

Histopathological main-findings:

No treatment-related changes were noted in the skin samples exposed to the 3 doses of Test Item. A representative H&E stained section from the higher dose (100µg/ml) is shown as compared to Negative Control (normal layers). In contrast, exposure to the Positive Control compound was associated with necrosis of the epidermal cells.