# Human Skin Model Test: *In Vitro* Skin Irritation

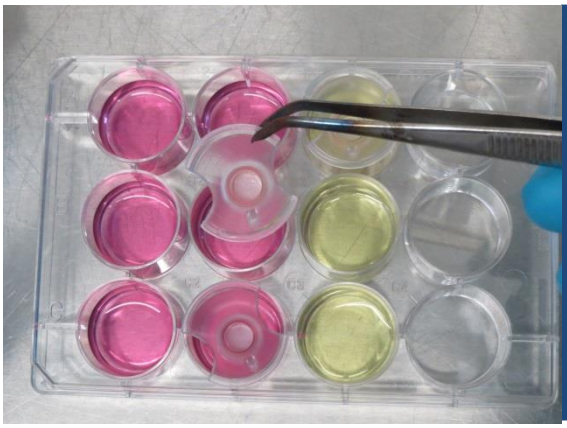
## (EpiDerm™ and EPISKIN-SM™)

The prediction and classification of skin irritation potential of substances can be performed by the assessment of the effect on EpiDerm™ and EPISKIN-SM™ models.

The human skin model test is validated by the EURL ECVAM (European Union Reference Laboratory for Alternatives to Animal Testing) and is performed in accordance with the OECD guidance OECD 439 at Eurofins BioPharma Product Testing Munich GmbH [1] with chemicals, cosmetics or personal care products and pharmaceuticals.

## Assessment of Skin Irritation Potential

- Acute irritation characterizes a local, reversible inflammatory response of normal living skin to direct injury after application of irritant substances.[2]
- The EpiDerm™ and EPISKIN-SM™ represent a reconstituted three-dimensional human epidermis (RhE) model which consists of human epidermal keratinocytes. This *in vitro* model mimics biochemical and physiological properties of the upper human skin.
- To determine skin irritation potential substances are applied directly on the skin tissue surface and the cell viability is measured by the MTT assay.
- The EpiDerm™ and EPISKIN-SM™ human skin model test can be used as a reliable *in vitro* method to identify substances as non-classified ("No Category") or they can be classified into UN GHS "Category 2". A positive result will further require a skin corrosion test (OECD 431) for a final classification into "Category 1". [1]
- The skin irritation potential of medical device extracts can also be determined by using a modified EpiDerm™ human skin model test.



**Acute irritation characterizes a local, reversible inflammatory response of normal living skin to direct injury after application of irritant substances [2]. After an incubation period, which is different for solids and liquids, the viability of the cells is measured by the MTT assay.**

## Procedure

Principles of the Human Skin Model Test

| Protocol | | |
|---|---|---|
| **Model** | EpiDerm | EpiSkin |
| **Supplier** | MatTek | Skin Ethic |
| **Analysis** | Skin irritation potential: cytotoxicity measurement with MTT (mean tissue viability compared to negative control) | Skin irritation potential: cytotoxicity measurement with MTT (mean tissue viability compared to negative control)<br>optional: Interleukin-1α (IL-1α) release into the tissue culture medium |
| **Test chemical concentrations** | Liquids: 30 µL (undiluted)<br>Solids: 25 mg + 25 µL DPBS | Liquids: 10 µL (undiluted)<br>Solids: 10 mg + 25 µL DPBS |
| **Exposure time and incubation periods** | 60 min incubation with dose groups<br>24 h post-incubation and further 18 h incubation in media | 15 min incubation with dose groups<br>42 h post incubation |
| **Quality controls** | Positive control: 5% SDS<br>Negative control: DPBS | |
| **Pre-tests** | **To determine if additional controls are needed:**<br>**NSMTT***: mixing of test item with MTT medium to determine if test item alone can reduce MTT → blue colouring: in main experiment two killed tissues treated with test item and two untreated killed tissues are added as controls<br>**$NSC_{living}$**[#]: mixing of test item with $H_2O$ or isopropanol to determine if strong colour of test item can discolour at contact with this liquids → optical discolouring (measuring of spectrum): in main experiment two living tissues without incubation with MTT medium are added as controls<br>**$NSC_{killed}$**[§]: if the two other controls were determined → in main experiment two killed tissues without incubation with MTT medium are added as controls | |
| **Application** | Direct topical application of chemicals on skin tissues<br>Three tissue replicates per dose group | |
| **Data delivery** | Optical density (OD) value with microplate spectrophotometer at 570 nm | |
| **Prediction model** | **Mean tissue viability (% negative control):**<br>≤ 50%: Irritant (I): UN GHS "Category 2" (IL-1α > 60 pg/mL)<br>> 50%: Non-Irritant (NI): UN GHS "No Category" (IL-1α ≤ 60 pg/mL) | |

*NSMTT: non-specific MTT reduction
[#]$NSC_{living}$: non-specific colouring of living tissues
[$]$NSC_{killed}$: non-specific colouring of killed tissues

## Data

Eurofins Data for demonstration technical proficiency of the Human Skin Model Test (EpiDerm™ and EPISKIN-SM™)

| Chemical | UN GHS category | EF category for EpiDerm™ | EF category for EPISKIN-SM™ |
|---|---|---|---|
| **Non-Classified Chemicals (UN GHS "No Category")** | | | |
| Naphthalene acetic acid | No Category | No Category | No Category |
| Isopropanol | No Category | No Category | No Category |
| Methyl stearate | No Category | No Category | No Category |
| Heptyl butyrate | No Category | No Category | No Category |
| Hexyl salicylate | No Category | No Category | No Category |
| **Classified Chemicals (UN GHS "Category 2")** | | | |
| Cyclamen aldehyde | Category 2 | Category 2 | Category 2 |
| 1-Bromohexane | Category 2 | Category 2 | Category 2 |
| 1-Methyl-3-phenyl-1-piperazine | Category 2 | Category 2 | Category 2 |
| Heptanal | Category 2 | Category 2 | Category 2 |

EF = Eurofins Munich GmbH

**Table 1:** Eurofins data of *in vitro* skin irritation with EpiDerm™ and EPISKIN-SM™ of nine tested proficiency chemicals compared to the data of the OECD guideline 439.[1]

In Table 1 the obtained data from the *in vitro* skin irritation with EpiDerm™ and EPISKIN-SM™ of five non-classified and four classified ("Category 2") chemicals are shown. The prediction of all tested chemicals was correct in comparison to the classification of the OECD guideline 439.

## References


1) OECD (2015). OECD Guideline for the Testing of Chemicals No. 439: *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method. 28 July 2015
2) UN (2015). United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), sixth revised edition, UN New York and Geneva