Human Skin Model Test with Medical Devices: *In Vitro* Skin Irritation

(EpiDerm™)

The prediction and classification of medical devices by the human skin model test of skin irritation potential can be done by the assessment of the effect on EpiDerm™ model.

The modified human skin model test for medical devices is a reliable *in vitro* test method and is performed in accordance with the draft ISO guideline 10993-10 Part 10 at Eurofins BioPharma Product Testing Munich GmbH.¹

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**Assessment of Skin Irritation Potential of Medical Devices**

- Acute irritation characterizes a local, reversible inflammatory response of normal living skin to direct injury after application of irritant substances.²

- The EpiDerm™ represents 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 the test item is extracted and applied directly on the skin tissue surface. The cell viability is measured by the MTT assay.

- The EpiDerm™ human skin model test can be used as a reliable *in vitro* test method to identify extracts from medical devices as "irritant" or "non-irritant".³

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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 of 18 ± 0.5 h the viability of the cells is measured by the MTT assay.
## Procedure

### Principles of the Human Skin Model Test with Medical Devices

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<th>Protocol</th>
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<td><strong>Model</strong></td>
<td>Human skin model EpiDerm™ supplied from MatTek</td>
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| **Analysis** | Skin irritation potential: cytotoxicity measurement with MTT (mean tissue viability compared to negative control tissues)  
Optional: Interleukin-1α release into the tissue culture medium |
| **Test chemical concentrations** | 100 µL undiluted extract in 0.9% NaCl (polar)  
100 µL undiluted extract in sesame oil (non-polar) |
| **Extraction and absorption capacity** | Extraction at 37 ± 1 °C for 72 ± 2 h in 0.9% NaCl or sesame oil with continuous agitation / shaking  
Determination of the absorption capacity of the test item in 0.9% NaCl (polar) or in sesame oil (non-polar) |
| **Exposure time** | 18 ± 0.5 h in the incubator |
| **Quality controls** | Positive control: 1% SDS in NaCl and sesame oil  
Negative control: DPBS  
Vehicle controls:  
→ 0.9% NaCl as solvent for polar extracts  
→ sesame oil as solvent for non-polar extracts |
| **Pre-tests** | **To determine if additional controls are needed:**  
**NSMTT**: mixing of test item extract 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 were added as controls  
**NSC<sub>living</sub>**: mixing of test item extract with H<sub>2</sub>O or isopropanol to determine if strong own 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 were added as controls  
**NSC<sub>killed</sub>**: if the two other controls were determined  
→ in main experiment two killed tissues without incubation with MTT medium were added as controls |
| **Application** | Direct topical application of extracts on skin tissues  
Three tissue replicates per dose group |
| **Data delivery** | Optical density (OD) value with microplate spectrophotometer at 570 nm  
Tissue viability of each dose group |
| **Prediction model** | **Mean tissue viability (%) negative control:**  
≤ 50%: Irritant; (Interleukin-1α > 60 pg/mL)  
> 50%: Non-Irritant; (Interleukin-1α ≤ 60 pg/mL) |

*NSMTT*: non-specific MTT reduction  
*NSC<sub>living</sub>*: non-specific colouring of living tissues  
*NSC<sub>killed</sub>*: non-specific colouring of killed tissues
References

1) ISO 10993-10, 2010(E), "Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization". Annex D: *In vitro* tests for skin irritation
