### Skin irritation protocol

**Episkin (validated)**

**Model:** EpiSkin™ Small Model

### DESCRIPTION

- **Culture insert equilibration period:** incubate for a 1 h up to 48 h (37°C)
- **Transfer tissues to fresh maintenance medium**
- **Treatment on 3 tissue replicates per condition:**
  - Liquids and viscous: 10 μL ± 2 μL
  - Solids: 5 μL H2O + 10 mg ± 2 mg
  - Waxy/sticky: 10 ± 2 mg with a nylon mesh
- **Treatment Period:** Incubate for 15 min (± 30 sec) at RT
- **Rinse with PBS+**
- **Incubate tissues in 0.3 mg/mL MTT solution for 3 h ± 15 min (37°C)**
- **Make a biopsy of the epidermis**
- **Extraction:** Immerse the tissue in 500 μL acidified isopropanol
- **Extract formazan minimum 4h at RT or 18h to 72h at +5°C**
- **Read OD with microplate spectrophotometer at 570 nm**

### PREDICTION MODEL

<table>
<thead>
<tr>
<th><strong>In vitro Result</strong></th>
<th><strong>Classification (In vivo Prediction)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean tissue viability ≤ 50%</td>
<td>Category 2 (previously R38), Irritant (I)</td>
</tr>
<tr>
<td>Mean tissue viability &gt; 50%</td>
<td>No Category (previously No label), Non Irritant (NI)</td>
</tr>
</tbody>
</table>