Skin irritation protocol
SkinEthic (validated)

Model: SkinEthic™ RHE

DESCRIPTION

Culture inserts equilibration period: Incubate at least 2 h (37°C)
↓
Transfer tissues to fresh medium
↓
Treatment: on 3 tissue replicates per condition:
  o Liquids and viscous: 16 μL ± 0.5 μL using a nylon mesh
  o Solids: 10 μL H2O + 16 ± 2 mg
  o Waxy/sticky: 16 ± 2 mg using a nylon mesh
↓
Treatment Period: Incubate for 42 min at RT
↓
Rinse thoroughly 25 times with 1 mL PBS
↓
Post Treatment Period: Incubate for 42 h 1 h (37°C)
↓
Incubate tissues in 1 mg/mL MTT solution for 3 h ± 15 min (37°C)
↓
Extraction: Transfer the inserts in 750 µL isopropanol, add 750 µL on the top of each tissue
↓
Extract formazan at least 2 h
↓
Perforate the insert and homogenize formazan extract
↓
Read OD with microplate spectrophotometer at 570 nm

PREDICTION MODEL

<table>
<thead>
<tr>
<th>In vitro Result</th>
<th>Classification (In vivo Prediction)</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>
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