

## 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:

Liquids and viscous: 16 μL ± 2 μL using a nylon mesh
Solids: 10 μL H2O + 16 ± 2 mg

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  $\pm$  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



<i>In vitro</i> Result	Classification ( <i>In vivo</i> Prediction)
Mean tissue viability ≤ 50%	Category 2 (previously R38), Irritant (I)
Mean tissue viability > 50%	No Category (previously No label), Non Irritant (NI)

