

SKIN IRRITATION PROTOCOL

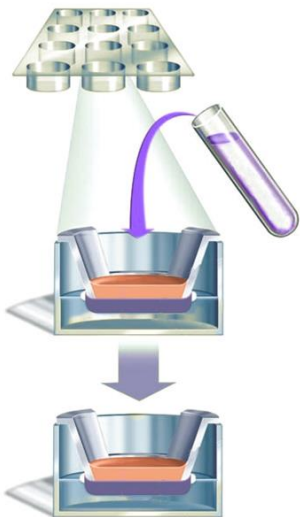
SkinEthic (validated)

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 ± 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

<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)