

Skin corrosion protocol SkinEthic (validated)

Model: SkinEthic™ RHE

DESCRIPTION

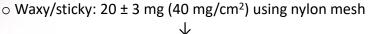
Culture insert equilibration period: incubate for 3 h up to 24 h (37°C)

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Transfer tissues to fresh maintenance medium



Treatment: At least 2 tissues per exposure time: • Liquids and viscous: $40 \mu L \pm 3\mu L$ ($80 \mu L/cm^2$) using nylon mesh • Solids: $20 \mu L \pm 2\mu L$ H₂O + 20 ± 3 mg (40 mg/cm^2)



Treatment Period: Incubate for 3 min and 60 min at RT



Rinse 20 times with a constant soft stream of 1mL PBS-



Incubate tissues in 1 mg/mL MTT solution for 3 h \pm 15 min (37° C)



Extraction: Immerse the inserts in 1.5 mL isopropanol (extraction from top and bottom of insert)



Extract formazan overnight without shaking at 4°C or for 120 min with shaking (~120 rpm) at RT



Perforate the insert and homogenize formazan extract



Read OD with microplate spectrophotometer at 570 nm

PREDICTION MODEL

≥ 18% after 3 min exposure

<i>In vitro</i> Result	Classification (<i>In vivo</i> Prediction)
STEP 1	
< 50% after 3 min exposure	Corrosive (C)
≥ 50% after 3 min exposure AND < 15% after 60 min exposure	Corrosive (C)
≥ 50% after 3 min exposure AND ≥ 15% after 60 min exposure	Non-corrosive
Step 2 for substances / mixtures identified as Corrosive	in step 1
< 18% after 3 min exposure	Optional Sub-category 1A (Cat. 1A)

Optional Sub-categories 1B-and-1C 1A (Cat. 1B-and-1C)