

SKIN CORROSION PROTOCOL

SkinEthic (validated)

EPISKIN

L'ORÉAL RESEARCH & INNOVATION

DFC-PRO017/01

Model

SkinEthic™ RHE

DESCRIPTION



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



Transfer tissues to fresh maintenance medium



Treatment: At least 2 tissues per exposure time:

- Liquids and viscous: 40 $\mu\text{L} \pm 3\mu\text{L}$ (80 $\mu\text{L}/\text{cm}^2$) using nylon mesh
- Solids: 20 $\mu\text{L} \pm 2\mu\text{L}$ H_2O + 20 ± 3 mg (40 mg/cm²)
- Waxy/sticky: 20 ± 3 mg (40 mg/cm²) using nylon mesh



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)



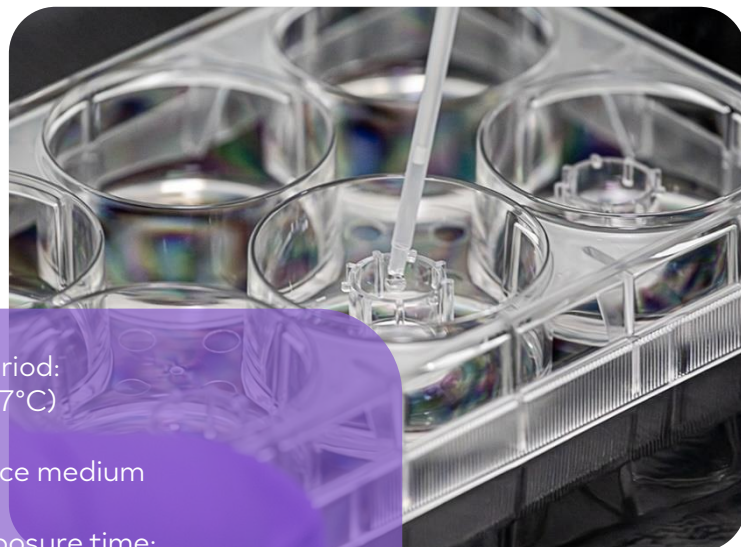
Extract formazan overnight without shaking at 4°C or for 120 min with shaking (~120 rpm) at RT



Perforate the insert and homogenize formazan extract



Read OD with microplate spectrophotometer at 570 nm



PREDICTION MODEL

<i>In vitro</i> Result	Classification (<i>In vivo</i> Prediction)
STEP 1	
< 50% after 3 min exposure	Corrosive (C)
$\geq 50\%$ after 3 min exposure AND < 15% after 60 min exposure	Corrosive (C)
$\geq 50\%$ after 3 min exposure AND $\geq 15\%$ 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)
$\geq 18\%$ after 3 min exposure	Optional Sub-categories 1B-and-1C 1A (Cat. 1B-and-1C)