

Eye irritation protocol

Solid raw materials

Model: SkinEthic™ HCE

DESCRIPTION

Receipt: Transfer epithelium from agarose to maintenance medium in 6-well plate and incubate at least overnight (37°C)



Treatment: 2 tissues each with 30 µL PBS- (Negative Control) or 30 µL methyl acetate (Positive Control) or 30 µL PBS- + 30 mg test chemical (test treatment)



Transfer tissues to fresh maintenance medium in the same 6-well plates



Treatment Period: Incubate for 4h ± 5min (37°C)



Rinse with PBS- (25mL: 2mL/ jet)



Post-soak Immersion: Immerse the tissue in 4 mL fresh maintenance medium, in 12-well plate
Post-soak Period: Incubate for 30 min ± 2min at RT



Transfer tissues to fresh maintenance medium in the same 6-well plates



Post-incubation Period: Incubate for 18h in t(37°C)



Viability: Transfer tissues into MTT solution (1mg/mL)



Incubate tissues for 3h cubate ti(37°C)



Extraction: place the inserts on 1.5mL isopropanol in 12-well plate



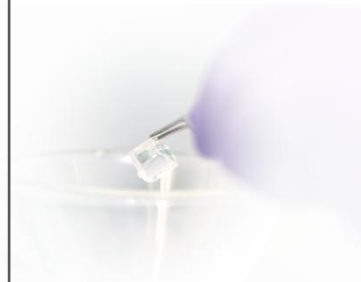
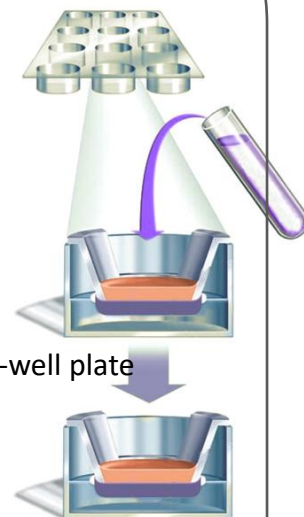
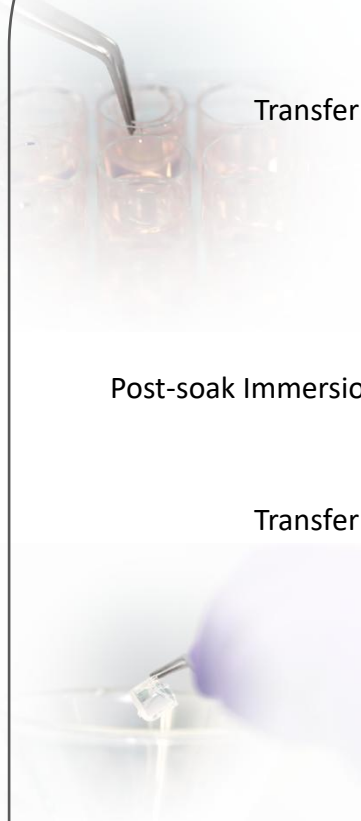
Extract formazan (2h at least, RT)



Remove 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%	No Category
Mean tissue viability ≤ 50%	Classified, Category 1 / Category 2