



# Eye irritation protocol

## Time-To-Toxicity test method

### 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)



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



Treatment: 2 tissues each with 80 µL PBS- (Negative Control, NgC) or 80 µL Lactic acid 1% in water (w/v) + 80 µL distilled water (Positive Control, PC) or 80 µg test chemical (test treatment) + 80 µL distilled water + mesh for mixed



Treatment Period: Incubate for 30 ± 2min (37°C) or 120 ± 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



Viability: Transfer tissues into MTT solution (1mg/mL) in 24-well plate



Incubate tissues for 3h ± 15min (37°C)



Rinse the MTT solution with PBS-

Transfer the insert into a well plate (dry) during overnight, 4°C or Perform the extraction directly



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



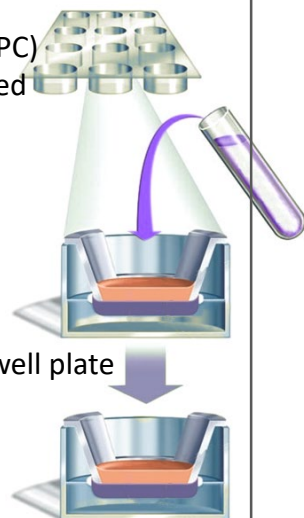
Extract formazan (2h at least, RT)



Remove the insert and homogenize formazan extract



Read OD with microplate spectrophotometer at 570 nm



#### PREDICTION MODEL

In vitro Result		Classification (In vivo Prediction)
Time treatment : 30min	Time treatment : 120min	
Mean tissue viability ≤ 40%	≤ 60%	Category 1
Any other combination of values		Category 2
> 40%	> 60%	No Category

