

EYE IRRITATION PROTOCOL

Solid raw materials

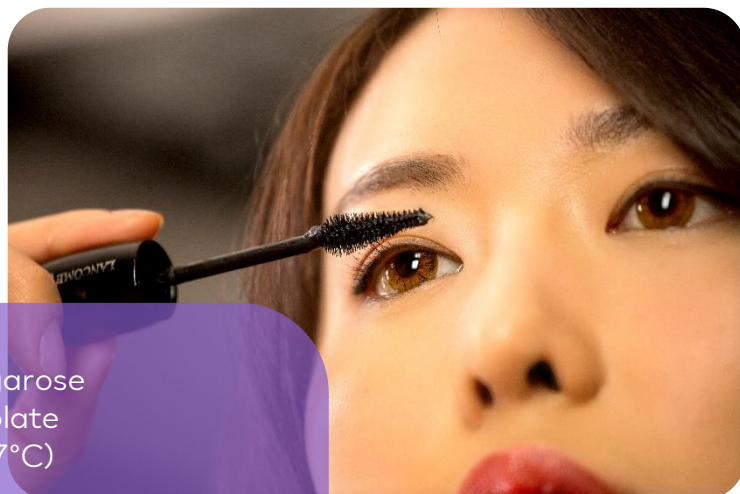
EPISKIN

L'ORÉAL RESEARCH & INNOVATION

DFC-PRO010/01

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 μ L \pm 2 μ L PBS- (Negative Control) or 30 \pm 2 μ L methyl acetate (Positive Control) or 30 μ L PBS- + 30 \pm 2mg test chemical (test treatment)



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



Treatment Period: Incubate for 4h \pm 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 \pm 2min at RT



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



Post-incubation Period: Incubate for 18h \pm 30 min (37°C)



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



Incubate tissues for 3h \pm 15 min (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

Criteria for <i>In vitro</i> interpretation	Classification
Mean tissue viability > 50%	No Category (NC)
Mean tissue viability \leq 50%	No prediction can be made