Eye irritation protocol
Time-To-Toxicity test method
Liquid raw materials

DESCRIPTION

Model: SkinEthic™ HCE

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

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

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Preparation of the 20% dilution (weight/volume) in distilled water of methyl acetate (Positive Control) and test chemicals for time treatment 16 and 120min

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Treatment: 2 tissues each with 80 µL PBS without Ca2+ & Mg2+ (Negative Control)
or 10µL PBS- + 80 µL methyl acetate (Positive Control)
or 10µL PBS- + 80 µL test chemical (test treatment)
Neat for 5min time treatment or 20% w/v for 16 and 120min time treatment

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Treatment Period: Incubate for 5min ± 15s (RT) or for 16min ± 1min (RT) or for 120min ± 2min (37°C)

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

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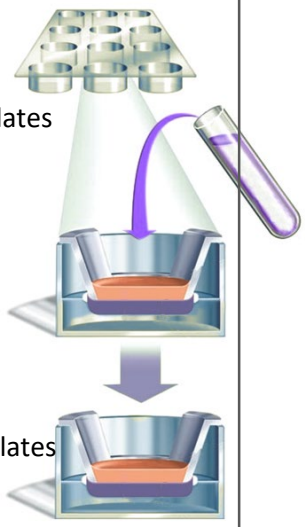
Post-Soak Immersion: Immerse tissues in 4 mL fresh maintenance medium in 12-well plates
Post-Soak Period: Incubate for 10min (RT)



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Viability: Transfer tissues into MTT solution in 24-well plates

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

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Rinse the MTT solution with PBS-

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Extraction: Immerse the inserts in 1.5mL isopropanol (formazan extraction) in 24-well plates

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Extract formazan (minimum 2h, RT or overnight, 4°C)

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Perforate the insert and homogenize formazan extract

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Read OD with microplate spectrophotometer at 570 nm

PREDICTION MODEL

In vitro Result			Classification (*In vivo* Prediction)
Test chemical neat	***Test chemical diluted at 20%***		
Time treatment: 5 min	***Time treatment: 16 min***	***Time treatment: 120 min***	
Mean tissue viability ≤ 50 %	≤ 50 %	≤ 50 %	Category 1
Any other combination of values			Category 2
> 50%	> 50%	> 50%	No Category

EPISKIN