

Eye irritation protocol Liquid raw materials

Model: SkinEthicTM HCE

DESCRIPTION

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 24-well plates

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Treatment: 2 tissues each with 30 μ L PBS without Ca2+ & Mg2+ (Negative Control) or 30 μ L methyl acetate (Positive Control) or 30 μ L test chemical (test treatment)

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

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Rinse with PBS- (20 mL: 10 mL/jet)

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Post-Soak Immersion: Immerse tissues in 1.5 mL fresh maintenance medium

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Post-Soak Period: Incubate for 30min oak Per(37°C)

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Viability: Transfer tissues into MTT solution

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

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Extraction: Immerse the inserts in 1.5mL isopropanol (formazan extraction)

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

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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)
Mean tissue viability > 60%	No Category
Mean tissue viability ≤ 60%	Classified, Category 1 / Category 2

