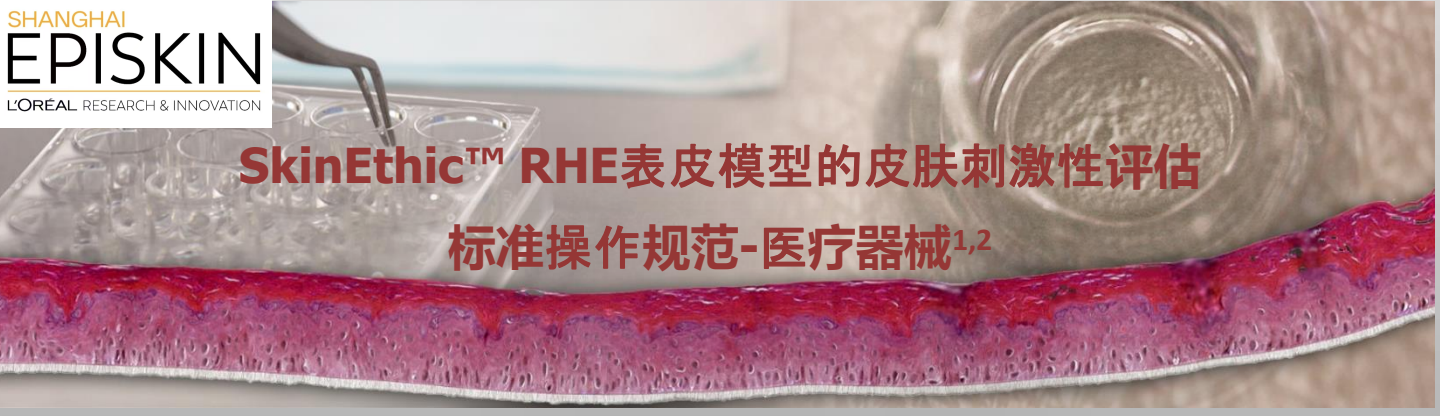
SHANGHAI EPISKIN
L'ORÉAL RESEARCH & INNOVATION



SkinEthic™ RHE表皮模型的皮肤刺激性评估
标准操作规范-医疗器械[1,2]

快速流程图

聚合物，聚氯乙烯

↓

萃取：按照ISO10993 -12标准，用极性溶剂（0.9% 氯化钠溶液）或非极性溶剂（药用级芝麻油）在37±1 ℃下萃取 72±2小时

↓

接收：将SkinEthic™ RHE表皮模型转移入6孔板的维持培养基中(每孔1毫升)

↓

平衡孵育: 过夜或至少2小时(37 ℃, 5% CO_2, 湿度 ≥90%)

↓

将表皮模型转移至6孔板的维持培养基（每孔1毫升）

↓

加样处理: 每个受试模型上加100±0.5微升的对照品或待测品
每组实验中，分别使用1个阴性对照，2个阳性，2个溶剂对照

- 阴性对照 ：PBS
- 阳性对照1：1% SDS 溶解于0.9% 的氯化钠溶液
- 阳性对照2：1% SDS 溶解于芝麻油溶液
- 溶剂对照1：0.9% 的氯化钠溶液
- 溶剂对照2：芝麻油

↓

处理时间：培养24±2小时（37±2 ℃，5±1% CO_2，湿度≥90% ）

↓

冲洗：25次，每次用1毫升PBS-(不含Ca^{2+}，Mg^{2+})

↓

细胞活性：模型转移至24孔板，每孔加入300微升MTT溶液（1毫克/毫升）
在37±2 °C下培养 3小时±15分钟

↓

提取：模型上下各加750微升异丙醇，室温避光条件下轻柔震荡孵育 2小时±5分钟

↓

读数：用酶标仪在570纳米波长下读取光密度值（OD值）

预测指标

体外检测标准	分类
至少一个样品提取物的平均组织活性 ≤ 50 %	刺激性 (I)
两个样品提取物的平均组织活性 > 50 %	非刺激性 (NI)

1- SkinEthic™ RHE for in vitro evaluation of skin irritation of medical device extracts. Pellevoisin C, Videau C, Briotet D, Grégoire C, Tornier C, Alonso A, Rigaudeau AS, Bouez C, Seyler N. Toxicol In Vitro. 2018 Aug;50:418-425. doi: 10.1016/j.tiv.2018.01.008. Epub 2018 Jan 13.

2 - Round robin study to evaluate the reconstructed human epidermis (RhE) model as an in vitro skin irritation test for detection of irritant activity in medical device extracts. De Jong WH, Hoffmann S, Lee M, Kandárová H, Pellevoisin C et al..Toxicol In Vitro. 2018 Aug;50:439-449. doi: 10.1016/j.tiv.2018.01.001. Epub 2018 Jan 8. No abstract available.

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SkinEthic™ RHE for *in vitro* evaluation of skin irritation of medical device extracts[1,2]

QUICK FLOW CHART

Polymers, PVC

↓

Extraction : Extract by polar solvent (0.9%NaCl) or non polar solvent (pharmaceutical grade sesame seed oil) for 72±2 h abide by ISO10993-12 (37±1°C)

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Receipt: Transfer SkinEthic™ RHE to maintenance medium in 6-well plate (1 mL/well)

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Culture: Overnight or at least 2 h (37°C, 5% CO_2, ≥90% humidity)

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Transfer tissue to maintenance medium in 6-well plate (1 mL/well)

↓

Treatment: 100±0.5 µL control or test substance

- Negative control : PBS
- Positive control 1: 1% SDS dissolved in 0.9%NaCl
- Positive control 2: 1% SDS dissolved in sesame oil
- Solvent control 1: 0.9%NaCl
- Solvent control 2: sesame oil

↓

Treatment Period : Incubate for 24±2 hours (37°C, 5% CO_2, ≥90% humidity)

↓

Rinsing: Rinse thoroughly 25 times with 1 mL PBS- (without Ca^{2+}, Mg^{2+})

↓

MTT incubation: Transfer tissues into MTT solution (1mg/mL) in 24-well plates (300µL MTT/well), incubate for 3 h±15 min (37±2°C)

↓

Extraction: Immerse the inserts in 1.5mL isopropanol (750 under and 750µL over in 24-well plate), incubate with gentle agitation for 2 h±5 min at RT protected from light

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Read OD with microplate spectrophotometer at 570 nm

PREDICTION MODEL

Criteria for *in vitro* interpretation	Classification
Mean tissue viability is ≤ 50% in at least one extraction vehicle	Irritant (I)
Mean tissue viability is > 50% in the two extraction vehicles	Non-Irritant (NI)

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