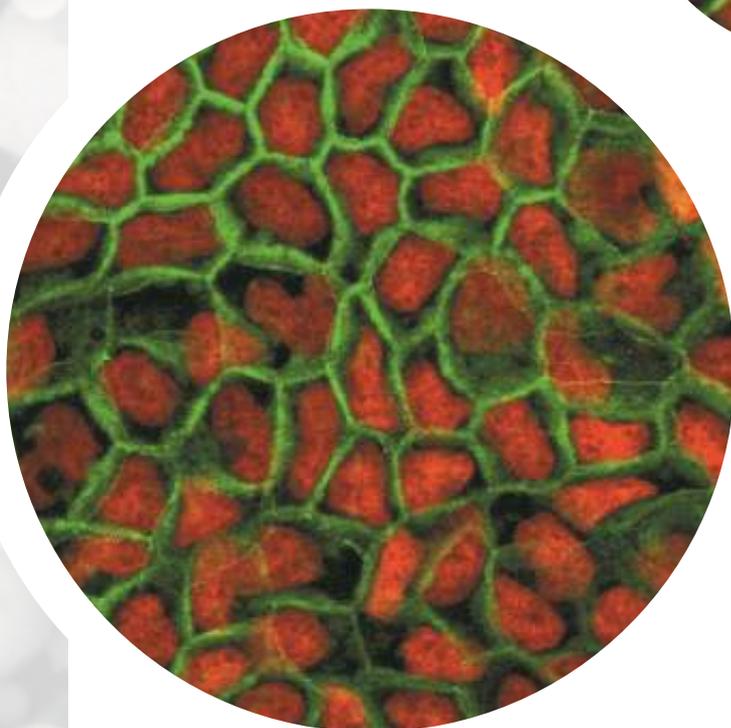
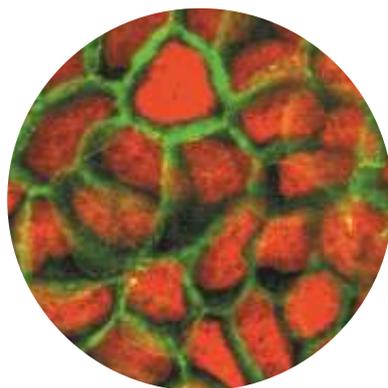


READYCELL



Safety and Toxicity
Custom Services

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Toxicity and Safety

Acute systemic toxicity

- OECD GD 129

BALB/c 3T3 Neutral Red Uptake Assay (3T3 NRU assay)
Cytotoxicity test (MTT test) in mouse 3T3 fibroblast cells.

Normal Human Keratinocyte Neutral Red Uptake Assay (NHK NRU assay)
Cytotoxicity test in Normal Human Keratinocytes.

Acute systemic toxicity assessment through different assays, on several species, in different cell types and systems. NRU, LDH release, MTT, WST-1, Resazurin test, ect. in human or animal primary cells or cell lines (kidney, liver, pancreas, intestinal, skin, etc.), among others, on demand.

- ECVAM report on Acute systemic toxicity (2002) and INVITTOX Protocol n° 51

LLC-RK1 Cell Test for Nephrotoxicity
Cytotoxicity, Barrier integrity (Transepithelial resistance, TEER) and paracellular permeability in LLC-PK1 (kidney proximal tubule cell line).

- ECVAM report on Acute systemic toxicity (2002) and INVITTOX Protocol n° 86

MDCK test for acute toxicity
Cytotoxicity, Barrier integrity (Transepithelial resistance, TEER) and paracellular permeability in MDCK (dog kidney epithelial cell line).

- ECVAM report on Acute systemic toxicity (2002) and INVITTOX Protocol n° 24

HepG2 Cell Test for Hepatotoxicity
Cytotoxicity, Protein content and Cell growth. Morphology and Cytoskeletal alterations, followed by Ph modifications in HepG2 liver cell line (hepatoma).

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Toxicity and Safety

Acute systemic toxicity

- INVITTOX Protocol nº 41

Chondrocyte functional toxicity test

Alteration analysis on Proteoglycans production by chondrocytes (Alcian Blue test) in Rabbit articular chondrocytes.

- ECVAM report on Acute systemic toxicity (2002)

Haematotoxicity test

Adenosine triphosphate (ATP) content, energy production and metabolism y HL-60 human acute promyelocytic leukemia cell line.

- INVITTOX Protocol nº 101

Haematotoxicity test for acute neutropenia

Colony Forming Unit-Granulocyte/Macrophage (CFU-GM) Assay in Human Cord Blood Mono Nuclear Cells (Hu-CBMNC) or Murine bone marrow Mono Nuclear Cells (MNC)



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Toxicity and Safety

Acute oral toxicity

- OECD guideline nº 425

Up and Down procedure

Animal survival rate, LD50, periodically clinical observations, body Weight and food/water consumption alterations, pathological analysis. The assay could be performed in different rodent species (rat preferred).

- OECD guideline nº 407

Repeat Dose 28-day

Daily clinical observations (health conditions, morbidity and mortality), Functional test (sensory reactivity test, motor activity, ect.). Body weight and food/water consumption alterations, Haematology, biochemical analysis, gross necropsy and Histopathology. This assay is performed in different rodent species.

- OECD guideline nº 420

Fixed Dose Procedure

Animal survival rate, periodically clinical observations, body Weight and food/water consumption alterations, pathological analysis. This assay could be performed in different rodent species (rat preferred).

- OECD guideline nº 423

Acute Toxic Class Method

Animal survival rate, periodically clinical observations, body Weight and food/water consumption alterations, performed in different rodent species (rat preferred).

Toxicity and Safety

Acute dermal toxicity

- OECD GD 129

Basal cytotoxicity test on skin cells

NRU, LDH release, MTT, WST-1, Resazurin test, ect. performed in Human skin primary cells or cell lines (Human epidermal progenitor cells, keratinocytes, dermal fibroblasts, melanocytes, HACAT, etc.), among others on demand.

- OECD guideline nº 402 (*in vivo*)

Acute dermal toxicity

Periodically clinical observations and pathological analysis in rat, rabbit or guinea pig.

- OECD guideline nº 410 (*in vivo*)

Repeated Dose Dermal Toxicity: 21/28-day Study

Daily clinical observations (health general conditions and toxicity signs), haematology, biochemical analysis, gross necropsy and histopathology in rat, rabbit or guinea pig.

- OECD guideline nº 411 (*in vivo*)

Subchronic Dermal Toxicity: 90-day Study

Daily clinical observations (health general conditions and toxicity signs), haematology, ophthalmological examination, biochemical analysis, gross necropsy and histopathology in rat, rabbit or guinea pig.

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Toxicity and Safety

Skin corrosion

- OECD guideline nº 430

In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TEER)

TEER measurement and Sulforhodamine B dye permeation analysis in rat skin discs.

- OECD guideline nº 431

In Vitro Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method

Cell Viability Measurements (MTT test) in Reconstructed Human Epidermis (RHE).

Optional: Histological analysis.

Skin irritation

- OECD guideline nº439

Reconstructed Human Epidermis (RHE) Test Method

Cell Viability Measurements (MTT test) in Reconstructed Human Epidermis (RHE).

Optional: Cytokine and inflammatory mediators release quantification and histological analysis.

- OECD guideline nº404 (*in vivo*)

Acute Dermal Irritation/Corrosion

Clinical observations and grading of the skin reaction (internal score) in albino rabbit.

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Toxicity and Safety

Ocular corrosives and severe irritants identification

- OECD guideline nº437

Bovine Corneal Opacity and Permeability Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage

Opacity (light transmission through the cornea) quantification using an Opacimeter and permeability of sodium fluorescein dye. Assay performed in Bovine Eye (Following selection criteria detailed on the OECD guideline).

- OECD guideline nº438

Isolated Chicken Eye Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage

Corneal opacity, swelling, fluorescein retention, and morphological effects performed in Chicken Eye (Following selection criteria detailed on the OECD guideline).

Optional: Photographs acquisition.

- OECD guideline nº460

Fluorescein Leakage Test Method for identifying Ocular Corrosives and Severe Irritants

Fluorescein permeability as an indicator of barrier function in MDCK dog kidney epithelial cell line.

Toxicity and Safety

Eye irritation

- INVITTOX protocol n°96

Hen's Egg Test on the Chorio-allantoic Membrane (HET-CAM)
 Macroscopical observation of coagulation, haemorrhage and lysis of blood vessels in the Chorio-allantoic Membrane in Hen's egg at day 10 after fertilisation.

- Protocol Reference Pending (Under prevalidation phase by ECVAM under a multicentric study)

Reconstructed Human Corneal Epithelium (RHCE) Test Method
 Cell Viability Measurements (MTT test) in Reconstructed Human Corneal Epithelium (RHCE)
 Optional: Cytokine and inflammatory mediators release quantification and histological analysis.



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Toxicity and Safety

Skin sensitization

- OECD guideline nº442A (*in vivo*)

Local Lymph Node Assay (LLNA): DA
Proliferation of lymphocytes in the lymph nodes of the animals, through the ATP content measurement by bioluminescence technique (Luciferase enzyme). Model: Mouse (CBA/J)

- OECD guideline nº442B (*in vivo*)

Local Lymph Node Assay (LLNA): BrdU-ELISA
Proliferation of lymphocytes in the lymph nodes of the animals, through the BrdU incorporation test. Model: Model: Mouse (CBA/J).

- OECD guideline nº406 (*in vivo*)

Skin sensitization
Clinical observations and grading of the skin reaction (internal score):
Erythema, swelling, etc. in Guinea pig.

Skin absorption

- OECD Guideline nº 428

Skin absorption in vitro method
Permeation and skin absorption of the test item through skin by chemical analytic techniques (UPLC/HPLC-UV, UPLC/HPLC-MS, HPLC-MS/MS, HPLC-QTOF, etc) in Human and pig skin biopsies.



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Toxicity and Safety

Phototoxicity

- OECD Guideline nº 101

UV-VIS ABSORPTION

UV-VIS absorption spectrum analysis of the test item by Spectrophotometric analysis.

- OECD Guideline nº 432

In vitro 3T3 NRU phototoxicity test

Photo-Irritation-Factor (PIF) or Mean Photo-effect (MEF) calculation. Classification of the product: No phototoxic, probable phototoxic or phototoxic. Model: 3T3 murine fibroblast cell line.

Mutagenesis

- OECD Guideline nº 471

Bacterial Reverse Mutation Test

Revertant colonies quantification (+/- S9). Data statistical analysis. Strain Models: *S. typhimurium* (TA1535, TA1537, TA97, TA97a, TA98, TA102 or TA100), *E. coli* (WP2 uvrA or WP2 uvrA (pKM101)).

- OECD Guideline nº 476

In vitro Mammalian Cell Gene Mutation Test

Cytotoxicity and viability determination, colony quantification and mutant frequencies calculation (+/- S9). Cellular models: L5178Y, CHO, AS52, V79 or TK6 cells.

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Toxicity and Safety

Genotoxicity

- OECD Guideline nº 487

In vitro Micronucleous Test (Mnvt)

Micronucleous quantification by fluorescence microscopy analysis (+/- Cyt. B) in Cultured primary human peripheral blood lymphocytes and cell lines (HL-60, CHO, L5178Y, etc.).



- ASTM-E2186: Standard Guide for Determining DNA Single-Strand Damage in Eukaryotic Cells Using the Comet Assay

COMET ASSAY in vitro

DNA damage rate (Percentage DNA tail) in Cultured primary human peripheral blood lymphocytes, hepatocytes, kupffer cells and cell lines (HL-60, CHO, L5178Y, etc.).

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