TEST CODE:
CT-003

# Skin Irritation Test

## RECONSTRUCTED HUMAN EPIDERMIS TEST METHOD (OECD TEST GUIDELINE 439)

## OVERVIEW

**Skin irritation is defined as reversible damage to the skin following exposure to a single substance or mixture (e.g. finished product) for up to 4 hours[1].**

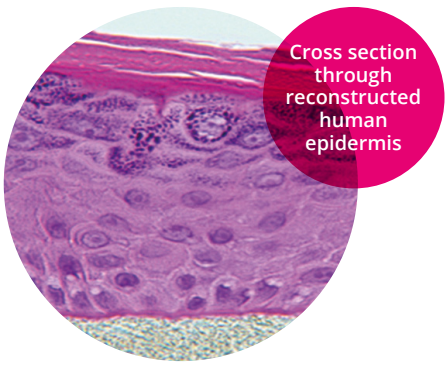
The test described here is fully accepted at a regulatory level for the hazard identification of irritant chemicals in accordance with the UN Globally Harmonized System (GHS) of Classification and Labelling Category 2. It can be used for single substances and for mixtures including finished products. It is appropriate for compliance with a range of legislation including REACH (Registration, Evaluation, Authorisation and restriction of CHemicals), the EU Cosmetics Regulation 1223/2009 and the CLP Regulation 1272/2008.

The test method is based on reconstructed human epidermis (RhE), which in its overall design mimics the biochemical and physiological properties of the upper parts of the human skin. The test item is applied directly to the skin surface, providing a good model of "real life" exposure. Cell viability is measured by enzymatic conversion of the vital dye MTT into a blue formazan salt that is quantitatively measured after extraction from the skin tissues. Irritant test items are identified by their ability to decrease cell viability below defined threshold levels (below or equal to 50% for UN GHS Category 2). If the viability is greater than 50%, the test item is classified as Non-Irritant ("no-label").

There are three validated skin models that adhere to this Test Guideline. XCellR8 can use the skin model of your choice or recommend the most appropriate model for your needs.

## TEST SYSTEM: RECONSTRUCTED HUMAN EPIDERMIS

Reconstructed human epidermis is a skin model composed of living human keratinocytes which have been cultured to form a multi-layered, highly differentiated epidermis. The levels of differentiation obtained are at the cutting edge of *in vitro* skin technology. The model consists of highly organized basal cells which progressively flatten out as the apical surface of the tissue is approached, analogous to the normal human *in vivo* epidermis. The model includes a functional skin barrier with an *in vivo*-like lipid profile. The profiles of key differentiation markers also mirror those seen *in vivo*. The cells are both metabolically and mitotically active, and release many of the pro-inflammatory agents (cytokines) known to be important in skin irritation and inflammation. Reconstructed human epidermis is grown on special platforms at the air-liquid interface, allowing for direct application of test items in a way that accurately models "real life" skin exposure.






**XCellR8 Ltd.** +44 (0)1925 607 134 | info@x-cellr8.com | www.x-cellr8.com
Techspace One, Sci-Tech Daresbury, Keckwick Lane, Daresbury, Cheshire, WA4 4AB, UK
Registered in England and Wales 6489519 | VAT number GB 932 3310 59.

CT-003/02-05/18

XCellR8 Redefining testing

TEST CODE:
CT-003

# Skin Irritation Test

## RECONSTRUCTED HUMAN EPIDERMIS TEST METHOD (OECD TEST GUIDELINE 439)

## SUMMARY OF THE TEST METHOD

- Skin models are pre-warmed in a cell culture incubator (37°C / 5% $CO_2$) for 60 minutes.
- The culture medium is replaced and the skin models are returned to the cell culture incubator for a further 18 hours.
- The test item is applied to the surface of the skin models: triplicate models are dosed at the apical surface with 30µl (liquid) or 25mg (solid).
- Controls consist of ultrapure water (negative control) and 5% SDS (positive control).
- The dosed skin models are placed into a cell culture incubator for 60 minutes.
- Test items and control substances are removed from the skin models' surface by washing.
- Skin models are returned to a cell culture incubator for a total recovery period of 42 hours. After 24 hours, if required, the culture medium may be saved for the additional analysis of other markers of inflammation and cell damage (including cytokines such as IL-1α).
- The viability of the skin models is assessed by MTT conversion. MTT solution is applied to the surface of the skin models and placed into a cell culture incubator for 3 hours. The blue formazan metabolite produced by viable cells is then extracted into isopropanol by incubation at room temperature for 2 hours.
- Triplicate samples of the extracted formazan solution are transferred to a microplate and the formazan product is quantified by absorbance spectrophotometry (wavelength 570nm).
- Absorbance readings of the formazan product from skin models incubated with test items are compared with those of negative controls to calculate percentage viability.
- A range of acceptance criteria must be satisfied in accordance with OECD TG439.
- If the viability is less than or equal to 50% after 60 minutes, the test item is classified as a Skin Irritant (UN GHS Category 2 / EU classification R38). If the viability is greater than 50%, the test item is classified as Non-Irritant ("no-label").

| | |
|---|---|
| TURNAROUND TIME | *6 – 8 weeks* |
| AMOUNT OF SAMPLE REQUIRED | *10ml (liquids) / 10g (solids)* |
| PRICE | *Our test prices are dependent on the quantity of test items. Please enquire for a quote using the contact information shown below, or the contact form on our website.* |
| FURTHER DOWNLOADS | OECD Test Guideline 439<br>XCellR8 Good Laboratory Practice (GLP) Compliance Certificate. |
| REFERENCES | *UN (2009), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Third revised edition, UN New York and Geneva.* |

### QUALITY STATEMENT

XCellR8 is accredited by the UK Medicines and Healthcare Products Regulatory Authority (MHRA) for the conduct of *in vitro* safety testing in compliance with Good Laboratory Practice (GLP). This means that we are able to provide our clients with test results that may be used at a regulatory level to demonstrate product safety, where the test is an approved regulatory method. The regulatory status is applicable in all global territories participating in the OECD's Mutual Acceptance of Data.

**XCellR8 Ltd.** +44 (0)1925 607 134 | info@x-cellr8.com | www.x-cellr8.com
Techspace One, Sci-Tech Daresbury, Keckwick Lane, Daresbury, Cheshire, WA4 4AB, UK
Registered in England and Wales 6489519 | VAT number GB 932 3310 59.



XCellR8 Redefining testing