TEST CODE:
CT-035

Skin Irritation Test

POTENCY ASSESSMENT METHOD

OVERVIEW

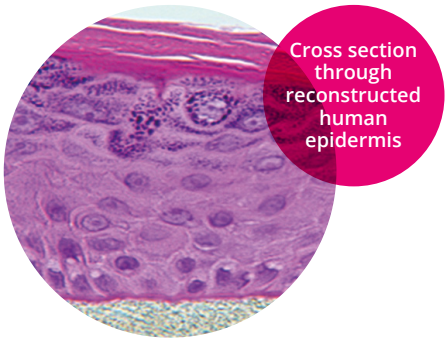
Skin irritation is defined as reversible damage to the skin following exposure to a single substance or mixture (eg finished product) for up to 4 hours[1].

The test described here is a non-regulatory method for the potency assessment of chemicals in terms of human skin irritation potential. It provides classification as a Severe, Moderate, Mild or Minimal / Non-Irritant and can be useful to classify a series of products or ingredients in rank order of skin irritation potential.

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. Irritation potential is calculated in terms of the "ET50" value: the time taken, in minutes, for the test item to reduce the viability of the skin model to 50%. ET50 values are then used to assign the irritancy classification based on the proven prediction model.

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.



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CT-035/02-05/18

XCellR8 Redefining testing

TEST CODE:
CT-035

Skin Irritation Test

POTENCY ASSESSMENT METHOD

SUMMARY OF THE TEST METHOD

- Skin models are pre-warmed in a cell culture incubator (37°C / 5% CO_2) for 60 minutes or overnight.
- 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 0.3% Triton X-100 (positive control).
- The dosed skin models are placed into a cell culture incubator for 2 hours, 5 hours and 18 hours, using triplicate models for each time point.
- Test items and control substances are removed from the skin models' surface by washing.
- 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 and controls are used to calculate the ET50 value.
- A range of acceptance criteria must be satisfied in order for the experimental run to be declared valid.
- A prediction model is used to convert the ET50 value to an equivalent *in vivo* human skin irritation potential. Test items are classified as Severe, Moderate, Mild or Minimal / Non-Irritants. Multiple test items can be ranked in order of skin irritation potential according to their ET50 values.

TURNAROUND TIME	*4 – 6 weeks*
AMOUNT OF SAMPLE REQUIRED	*Minimum 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	XCellR8 Good Laboratory Practice (GLP) Compliance Certificate.
REFERENCES	*[1]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 you with test results that may be used at a regulatory level to demonstrate product safety, where the test is an approved regulatory method. The test method described here is non-regulatory but is conducted in our GLP-accredited laboratory.

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