TEST CODE:
CT-005

Eye Irritation Test

POTENCY ASSESSMENT METHOD (ET50)

OVERVIEW

Eye irritation is defined as changes in the eye following the application of a test chemical to the surface, which are fully reversible within 21 days of application.[1]

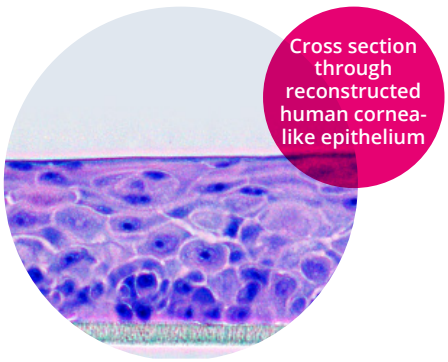
The test described here is a non-regulatory method for the potency assessment of chemicals in terms of human eye irritation potential. It provides classification as a Severe, Moderate, Mild or Minimal / Non-Irritant and can be useful to classify a series of products or ingredients in rank order of eye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

The test is based on the depth of injury model or Maurer and Jester, using the rationale that the degree of irritation caused by a test substance correlates with the degree of penetration into cell layers and subsequent impact on cell viability.

The method utilises reconstructed human cornea-like epithelium (RhCE), which in its overall design mimics the biochemical and physiological properties of the corneal epithelium of the human eye. The test item is applied directly to the cornea 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 CORNEA-LIKE EPITHELIUM

Reconstructed human cornea-like epithelium (EpiOcular™) is a corneal model composed of living human cells which have been cultured to form a multi-layered, differentiated corneal epithelium. The levels of differentiation obtained are at the cutting edge of *in vitro* tissue 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* corneal epithelium. 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 eye irritation and inflammation. EpiOcular™ 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" eye exposure.



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XCellR8 Redefining testing

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SUMMARY OF THE TEST METHOD

- EpiOcular™ models are pre-warmed in a cell culture incubator (37°C / 5% CO_2) for 60 minutes or overnight. The culture medium is replaced prior to applying treatment.
- A 20% solution of the test item is prepared by 1:4 dilution in ultrapure water.
- The test item is applied to the surface of the EpiOcular™ models: triplicate models are dosed at the apical surface with 100µl.
- 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 16 minutes.
- Test items and control substances are removed from the skin models surface by washing.
- Following a post-exposure soak in culture medium, the viability of the EpiOcular™ models is assessed by MTT conversion. MTT solution is applied to the surface of the 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 EpiOcular™ models incubated with test items are compared with those of negative controls to calculate percentage viability.
- The value obtained from this initial 16 minute incubation with test substance determines the time points for 2 further incubations, which are then carried out using a repeat of the above process. Time points are determined as follows. If the viability is >90% after the initial 16 minute exposure, subsequent time points are 64 and 256 minutes. If the viability is <90% but >30% after the initial 16 minute exposure, subsequent time points are 4 and 64 minutes. If the viability is <30% after the initial 16 minute exposure, the subsequent time points are 1 and 4 minutes. Triplicate positive controls (0.3% Triton X-100) are incubated for 4, 15 and 45 minutes.
- Absorbance values from all 3 time points are used to calculate ET50 (the time, in minutes, taken to reduce the viability of the EpiOcular model to 50% of the negative control value). The ET50 for the positive control should fall between 12.2 and 37.5 minutes.
- A statistical model is then used to convert the ET50 value to an equivalent *in vivo* Draize score.
- Draize scores are converted to eye irritation potential according to accepted classification criteria as follows: Draize score 0-15: Minimal / Non-Irritant. Draize score 15.1-25: Mild Irritant. Draize score 25.1-50: Moderate Irritant. Draize score 50.1-110: Severe / Extreme Irritant.
- A range of acceptance criteria must be satisfied in order for the experimental run to be valid.

TURNAROUND TIME	*5 – 7 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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