

TEST CODE:  
CT-049

# Mildness Test (Eye)

RECONSTRUCTED HUMAN CORNEA-LIKE EPITHELIUM METHOD (ET50)

## OVERVIEW

The reconstructed human cornea-like epithelium ET50 method is a powerful tool for assessing the mildness of cosmetic ingredients or formulations in contact with the eye. Using an adaptation of a regulatory eye irritation test, subtle differences between mild and ultra-mild formulations can be measured, allowing a series of products to be placed into a rank order of irritation potential (and therefore mildness). The data can be used to drive key decisions in formulation development, as well as providing valuable comparisons with industry benchmark products.

The ET50 method is commonly used to assess new baby formulations such as shampoos, foam baths and body washes, providing critical insights into the mildness of the product and comparisons with leading brands. The test provides a quick and cost-effective pre-screen, prior to human volunteer studies, providing a range of commercial and ethical benefits and enabling selection of the mildest formulations at an early stage of product development.

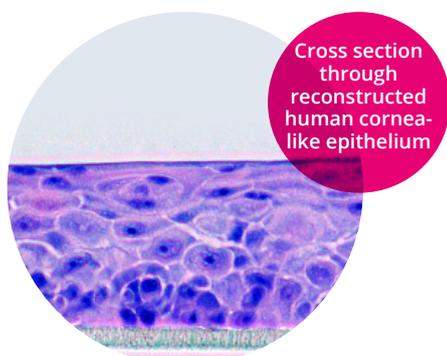
It also provides supporting information for safety claims such as "suitable for sensitive eyes". (Note that, in the absence of a definitive regulatory framework for the application of *in vitro* data in claim support, each ingredient or formulation must be carefully considered on a case-by-case basis. The results must always be applied in compliance with relevant local regulations and advertising standards).

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 corneal 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 corneal model to 50%. ET50 values are used to assign the irritancy classification (Severe, Moderate, Mild or Minimal / Non-Irritant) based on the validated prediction model and, where appropriate, to place the ingredients or products into a rank order of mildness.

## TEST SYSTEM

Reconstructed human cornea-like epithelium 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. Reconstructed human cornea-like epithelium 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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## SUMMARY OF THE TEST METHOD

- Corneal models are pre-warmed in a cell culture incubator (37°C / 5% CO<sub>2</sub>) 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 corneal models: triplicate models are dosed at the apical surface with 100µl liquid or 200mg of a 1:1 mix of solid with ultrapure water.
- Controls consist of ultrapure water (negative control) and 0.3% Triton X-100 (positive control).
- The dosed corneal models are placed into a cell culture incubator for 16 minutes.
- Test items and control substances are removed from the corneal models surface by washing.
- Following a post-exposure soak in culture medium, the viability of the corneal 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 corneal 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. Note: for ultra-mild formulations, a modified protocol is available, including exposure up to 24 hours.
- 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 15 to 45 minutes.
- A validated statistical model is then used to convert the ET50 value to eye irritation potential according to accepted classification criteria as follows: ET50 >256 - 26.5 minutes: Minimal / Non-Irritant. ET50 <26.5 - 11.7 minutes: Mild Irritant. ET50 <11.7 - 3.45 minutes: Moderate Irritant. ET50 <3.45 minutes: Severe / Extreme Irritant. Where relevant, test items are then placed into a rank order of mildness.
- A range of acceptance criteria must be satisfied in order for the experimental run to be valid.

COSMETIC CLAIMS SUPPORTED

Mild, "No Tears", Suitable for sensitive eyes

TURNAROUND TIME

6 - 8 weeks

AMOUNT OF SAMPLE REQUIRED

10ml (liquids) / 10g (solids). Please enquire if sample availability is limited.

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.](#)

## 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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