TEST CODE:
CT-048

Mildness Test (Skin)

EPIDERM™ METHOD (ET50)

OVERVIEW

The EpiDerm™ ET50 method is a powerful tool for assessing the mildness of cosmetic ingredients or formulations in contact with the skin. Using an adaptation of a regulatory skin irritation test, subtle differences between mild and ultra-mild formulations can be measured, allowing a series of products to be placed into rank order of irritation potential (and therefore mildness). It provides a quick and cost-effective pre-screen, prior to human volunteer studies, driving key decisions in formulation development and providing valuable comparisons with industry benchmark products.

The test 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.

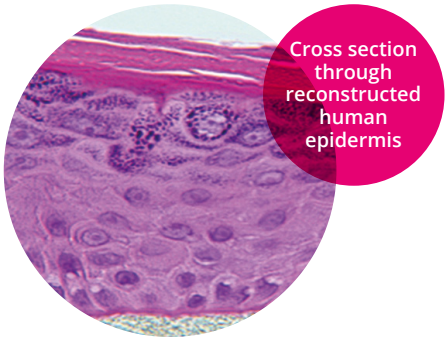
The ET50 test also applies to cosmetic ingredients, such as assessment of the mildness of novel "mild" surfactants against classic surfactants, replacing the traditional tests (such as HET-CAM and RBC) which often lack relevance and the required level of sensitivity.

The method can provide supporting information for safety claims such as "suitable for sensitive skin". (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 test method is based on reconstructed human epidermis (RhE), which mimics the biochemical and physiological properties of 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 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 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-048/02-05/18

XCellR8 Redefining testing

TEST CODE:
CT-048

Mildness Test (Skin)

EPIDERM™ METHOD (ET50)

SUMMARY OF THE TEST METHOD

- Skin models are pre-warmed in a cell culture incubator (37ºC / 5% CO2) 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.

COSMETIC CLAIMS SUPPORTED	*Mild, Suitable for sensitive skin*
TURNAROUND TIME	*6 – 8 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.

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.

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