TEST CODE:
CT-065

Gingival Irritation Test

ET50 METHOD

OVERVIEW

***In vitro* tissue models produced from primary human gingival cells can be used to evaluate the irritancy potential of oral care products and other relevant formulations that may come into contact with the gingival mucosa.**

The test method is based on primary human gingival cells that have been cultured to form a highly differentiated 3D model of human gingival mucosa. Products (test items) are applied directly to the tissue 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 tissues. Irritation potential is calculated in terms of the "ET50" value: the time taken, in minutes, for the product to reduce the viability of the tissue model to 50%.

ET50 values are then used to assign the rank order of irritation potential for a series of products or ingredients. As well as the ET50 determination, histological changes and cytokine expression may be assessed using the system. Additionally, inflammation may be induced within the test system using pro-inflammatory cytokines to investigate the protective or anti-inflammatory actions of test items.

TEST SYSTEM

The gingival epithelium is comprised of primary human gingival cells that have been cultured to form a highly differentiated 3D model of human gingival mucosa. The levels of differentiation obtained are at the cutting edge of *in vitro* tissue technology. The model consists of *Stratum Corneum*, granular layer, spinous layer and highly organized basal cells. Morphologically, the gingival epithelium is very similar to native gingival mucosa and profiles of key differentiation markers also mirror those seen *in vivo*. The tissues contain a *Stratum Corneum* unlike the oral epithelium model. The tissue models are grown on special platforms at the air-liquid interface, allowing for direct application of test items in a way that accurately models "real life" gingival exposure.

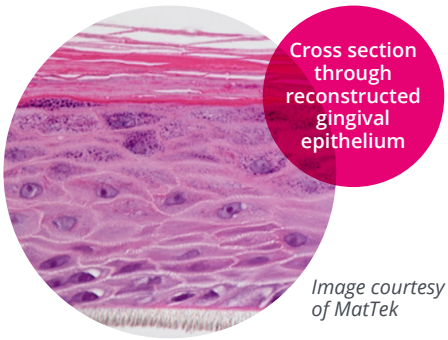

Image courtesy of MatTek

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.

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XCellR8 Redefining testing

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SUMMARY OF THE TEST METHOD

- Tissue models are pre-warmed in a cell culture incubator (37°C / 5% CO2) for 60 minutes or overnight.
- Test items are assessed by applying 100µl undiluted test item to the top of the EpiGingival tissue models for 3 exposure times, along with a negative control (undosed) \and positive control (1% Triton X-100).
- Test item dosing times are 1, 4 and 18 hrs.
- Negative control dosing time is 4hrs.
- Positive control dosing times are 5 and 18hrs.
- Test items and control substances are removed from the tissue model surface by rinsing.
- 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-6).
- The viability of the tissue models is assessed by MTT conversion. MTT solution is applied to the surface of the tissue 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 tissue models incubated with test items and controls are used to calculate the % tissue viability and the ET50 value.
- A range of acceptance criteria must be satisfied in order for the experimental run to be declared valid.
- Multiple test items can be ranked in order of their gingival irritation potential according to their ET50 values and compared against benchmark products or ingredients.

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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