TEST CODE:
CT-064

Oral Irritation Test

ET50 METHOD

OVERVIEW

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

The test method is based on normal, human-derived buccal cells that have been cultured to form a highly differentiated 3D model of human oral tissue. Finished products or ingredients 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 oral epithelium is comprised of normal, human-derived buccal cells that have been cultured to form a highly differentiated 3D model of human oral tissue. The levels of differentiation obtained are at the cutting edge of *in vitro* tissue technology. The model consists of highly organized basal cells, *Stratum Filamentosum* and *Stratum Distendum*. Morphologically, the oral epithelium is of uniform thickness and is very similar to native buccal tissue. The profiles of key differentiation markers also mirror those seen *in vivo*. The buccal tissues are non-keratinized epithelia that do not contain a granular layer or *Stratum Corneum* normally found in skin or gingival epithelia. 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" oral exposure.

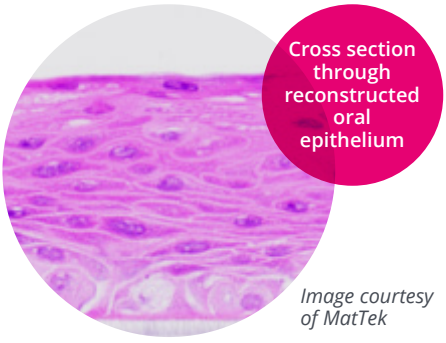

Image courtesy of MatTek

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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 diluted 1:1 in tissue culture grade sterile H2O. Then 40µl of the 1:1 diluted solution is added to the cell culture insert on top of the EpiOral tissue and applied for 3 exposure times, along with a negative control (tissue culture grade sterile H2O) and positive control (1% Triton X-100).
- Test item dosing times: Either 20, 60 and 120 minutes (standard test items) or 1, 4 and 18 hours (very mild materials).
- Negative control dosing time (median time for test items i.e. 60 mins or 4hrs).
- Positive control dosing times (30 and 120 mins).
- 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 oral 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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