TEST CODE:
CT-044

AcutoX Acute Toxicity Test

NEUTRAL RED UPTAKE METHOD

OVERVIEW

XCellR8's GLP-accredited laboratory provides the first truly animal-free preliminary screen for human acute oral toxicity. The AcutoX test is ideal as a rapid, cost-effective early screen in product development and to generate supporting information in a weight-of-evidence approach to safety assessment.

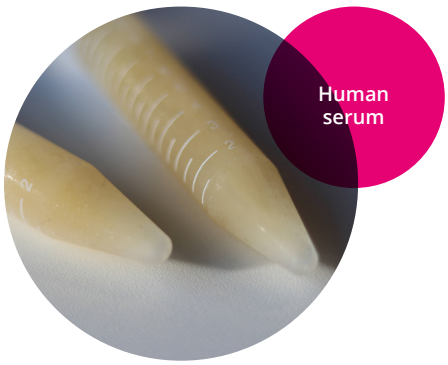
Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time (usually less than 24 hours).

Our test is a non-regulatory human cell-based method, using human dermal fibroblasts in animal product-free culture conditions, and assesses cell membrane damage (Neutral Red Uptake method) as an endpoint. The output of the test is an IC50 value (time taken to reduce the viability of the cells by 50%) as an *in vitro* alternative to the traditional animal-based *in vivo* LD50. A prediction model is used to place test items into predicted GHS classifications for human acute oral toxicity (category 3, 4 or 5).

It has been internally validated using a panel of 20 well-defined cosmetic ingredients.

TEST SYSTEM: HUMAN DERMAL FIBROBLASTS

The test system comprises of human dermal fibroblasts that are incubated for 24hrs with test items and controls prior to endpoint measurement with Neutral Red. The human dermal fibroblast cultures used in this test are obtained commercially as cryopreserved primary cells. They are originally derived from donor tissue (for example, following plastic surgery) with informed consent for the tissue to be used for research purposes, in adherence with the Human Tissue Act (UK) 2004. The cells have been extensively QC tested for a range of parameters including viability upon thawing from cryopreservation, proliferation rate, morphology and sterility (absence of bacteria, fungal growth and mycoplasma). They have also tested negative for HIV-1, HIV-2, HBV and HCV. They are maintained in the exponential growth phase in routine culture at 37°C / 5% CO2 prior to seeding into test plates. The culture medium and all culture reagents are free of animal-derived components, providing a fully human cell culture system.



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XCellR8 Redefining testing

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NEUTRAL RED UPTAKE METHOD

SUMMARY OF THE TEST METHOD

Range Finding Experiment

- Human dermal fibroblasts are seeded into 96 well plates and cultured for 24hrs under standard culture conditions (37°C, 5% CO2 and ≥95% relative humidity).
- Following this, the medium is removed and the cells are dosed with test items and controls.
- The positive control used is Sodium Dodecyl Sulphate (SDS).
- The test items are dosed using a wide dilution series (2 fold dilutions) based upon the highest soluble concentration.
- After a 24hr incubation with the test item, the cells are washed and then incubated with Neutral Red stain, which is incorporated into live cells.
- The IC50 value is calculated for the test item and is used to set the dosing range for the main experiment.
- One replicate is used to determine the appropriate dosing range.
- A range of acceptance criteria must be met in order to confirm validity of the test.

Main Experiment

- Setup and acceptance criteria are identical to the range finding experiment, with the exception of the test item dosing range.
- This is based around the range finding experiment IC50 value and a narrower series of concentrations is used to further hone-in on the IC50 value .
- Multiple replicates are used to demonstrate consistency of results.
- The IC50 values derived are used to identify the GHS category of the test item using the prediction model developed for the assay.
- The prediction model can sub-categorise into GHS Category 3 (Toxic if swallowed), 4 (Harmful if swallowed) or 5 (Potentially harmful if swallowed).

TURNAROUND TIME	*4 – 6 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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