TEST CODE:
CT-091

AcutoX

HUMAN-RELEVANT *IN VITRO* SCREEN FOR ACUTE ORAL TOXICITY

OVERVIEW

Acute toxicity describes the adverse effects of a substance resulting from single or multiple exposures in a short period of time (usually less than 24 hours) via the oral, dermal or inhalation routes. Traditionally, acute toxicity has been assessed in animals using LD50 type methods, which have been widely discredited both scientifically and ethically.

AcutoX is a human-relevant, animal-product-free *in vitro* test for acute oral toxicity. It is a rapid, cost-effective screen and generates supporting information in a weight-of-evidence approach to safety assessment. It can be used in combination with *in silico* approaches such as OPERA.

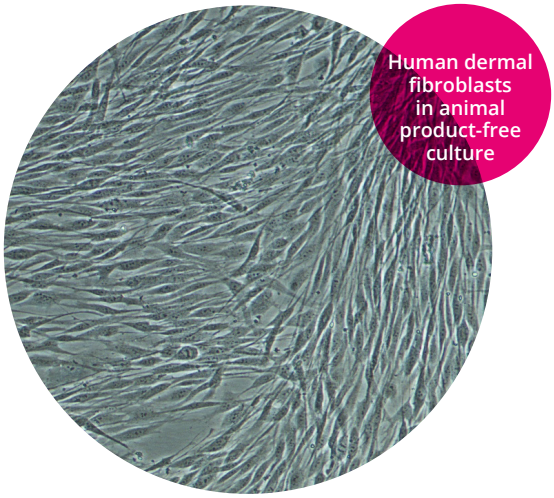
AcutoX uses human dermal fibroblasts and assesses cell membrane integrity (Neutral Red Uptake method) and cell metabolism (MTT method) as endpoints. The test includes pooled human liver extract (S9) to examine the toxifying or detoxifying effect of metabolism on a test item. The output of the test is an EC_{50} (the dose of cytotoxic test item at which 50% viability is achieved) as an *in vitro* alternative to the traditional animal-based *in vivo* LD50. AcutoX has been internally validated using a panel of nearly 70 test items, with a range of chemistries and from a variety of industry sectors. The validation process led to the development of a prediction model to assign a "toxic" or "non-toxic" category for both EPA and GHS classification systems, which can be further refined to assign a hazard category.

TEST SYSTEM: HUMAN DERMAL FIBROBLASTS

The test system consists of human dermal fibroblasts. The culture medium and all reagents are free of animal-derived components, providing a fully human *in vitro* system.

The cells are incubated with test items and controls prior to endpoint measurement with Neutral Red and MTT. 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% CO_2 prior to seeding into test plates.

XCellR8's animal product free lab


Human dermal fibroblasts in animal product-free culture

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XCellR8 Redefining testing

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HUMAN-RELEVANT *IN VITRO* SCREEN FOR ACUTE ORAL TOXICITY

SUMMARY OF THE TEST METHOD

Range finding experiment (with metabolism)

- Human dermal fibroblasts are seeded into 96 well plates and cultured for 24hrs in a humidified tissue culture incubator set to 37°C and 5% CO_2.
- Media is removed and the cells are dosed with test items and controls.
- The positive control used is 2-Butyne-1,4-diol.
- The test items are dosed using a wide dilution series (10-fold dilutions) in the presence of metabolically active liver extract (S9) and the relevant co-factors for 3 hours, based upon the highest soluble concentration.
- Cells are washed and incubated for a further 21 hours.
- After a 24hr (total) incubation, the cells are washed and then incubated with Neutral Red stain, which is incorporated into live cells.
- The EC_{50} 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 dosing range and several acceptance criteria must be met in order to confirm validity of the test.

Range finding experiment (without metabolism)

The Range finding experiment without metabolism follows the same protocol as above, save the following deviations.

- Positive control Chlorpromazine Hydrochloride is used.
- The cells are incubated with the test items for 24 hours.

Main Experiment (with metabolism)

- Human dermal fibroblasts are seeded into 96 well plates and cultured for 24hrs in a humidified tissue culture incubator set to 37°C and 5% CO_2.
- Media is removed and the cells are dosed with test items and controls.
- The positive control used is 2-Butyne-1,4-diol.
- The test items are dosed using a 2-fold dilution based around the EC_{50} calculated in the range finding experiment, in the presence of metabolically active liver extract (S9) and the relevant co-factors for 3 hours.
- Cells are washed and incubated for a further 21 hours.
- After a 24hr (total) incubation, the cells are washed and then incubated with Neutral Red stain, which is incorporated into live cells and MTT, which provides a readout of cellular metabolic health.
- The cell viability and an EC_{50} are calculated.
- Multiple replicates are used to demonstrate consistency.
- EC_{50} values derived from this assay are used to identify EPA and GHS classification, and chemicals that produce a similar EC_{50}.

Main Experiment (without metabolism)

The main experiment without metabolism follows the same protocol as above, save the following deviations.

- Positive control Chlorpromazine Hydrochloride is used.
- The cells are incubated with the test items for 24 hours.

TURNAROUND TIME	*6 – 10 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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