TEST CODE:
CT-012

Cytotoxicity Test

COMBINED MTT AND NEUTRAL RED UPTAKE METHOD

OVERVIEW

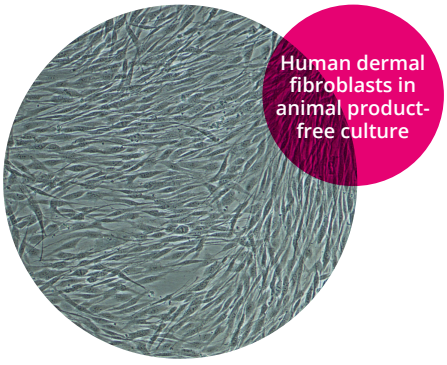
Cytotoxicity is defined as the degree to which a test item causes damage (toxicity) to cells. This may occur by one or more mechanisms including reduced metabolic capacity or damage to cell membranes. Cytotoxicity within a cell culture may be due to a reduced number of viable cells, or to reduced functional capacity of individual cells.

The test described here is a non-regulatory method for the assessment of cytotoxicity in terms of both metabolic capacity (MTT test) and membrane damage (Neutral Red Uptake - NRU). While the MTT and NRU tests can be provided separately if required, the combination of two key endpoints provides comprehensive information about the cytotoxic potential of a test item and the mechanism(s) involved. The method provides percentage viability values relative to untreated controls and an EC50 value (EC50 is the concentration of test item required to reduce the viability of the cells to 50%). The test can be useful to classify a series of products or ingredients in rank order of cytotoxic potential.

The method utilises human dermal fibroblasts in animal product-free culture conditions. This test system provides both scientific and ethical advantages of using a fully human cell based system to predict human toxicity. The test item is prepared in cell culture medium. If required for solubility purposes, DMSO may be present in the test up to a final concentration of 1%, provided that solvent controls are included. The test item is serially diluted to six test concentrations, and six replicates of each concentration are tested. Metabolic capacity is measured by enzymatic conversion of the vital dye MTT into a blue formazan salt that is quantitatively measured after extraction with isopropanol. Membrane integrity is measured by uptake of the vital dye Neutral Red into the cells.

TEST SYSTEM: HUMAN DERMAL FIBROBLASTS

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. The culture medium and all culture reagents are free of animal-derived components, providing a fully human cell culture system.



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CT-012/03-03/19

XCellR8
Redefining testing

TEST CODE:
CT-012

Cytotoxicity Test

COMBINED MTT AND NEUTRAL RED UPTAKE METHOD

SUMMARY OF THE TEST METHOD

- Human dermal fibroblasts are seeded into 96-well culture plates and incubated for 24± 2 hours at 37°C / 5% CO_2.
- Test items are prepared in culture medium by serial dilution to 6 test concentrations. If required for solubility purposes, DMSO is used to prepare a stock solution which is then diluted into culture medium. The final DMSO concentration does not exceed 1%.
- Controls consist of culture medium (negative control) and sodium lauryl sulfate (positive control). For test items requiring preparation in DMSO (see above), solvent controls (1% DMSO in culture medium) are also included.
- Test items and controls are applied to the plates, which are incubated at 37°C / 5% CO_2 for 24 hours (NRU) or 48 hours (MTT).
- Test items and control substances are removed from the cell culture plates by washing with Hanks' Balanced Salt Solution (HBSS).
- The viability of the cell culture is assessed by MTT conversion. MTT solution is applied to the plates and placed into a cell culture incubator for 3 hours. The blue formazan metabolite produced by viable cells is then extracted into isopropanol and absorbance is measured at 570nm using a spectrophotometer.
- The viability of the cell culture is assessed by Neutral Red Uptake (NRU). Neutral Red Solution is applied and the plates are placed into a cell culture incubator for 3 hours. The Neutral Red Solution is then removed and the cells are washed 3 times with Hanks Balanced Salt Solution (HBSS). Any Neutral Red that has been taken up and retained by the cells is then solubilised by the addition of Neutral Red Solubilisation Solution and the plates are placed on an orbital plate shaker for 30 minutes at room temperature. Neutral Red is quantified by absorbance at 540nm using a spectrophotometer.
- Absorbance values are used to calculate percentage viability values relative to the untreated negative controls. The EC50 value is also calculated (EC50 is the concentration of test item required to reduce the viability of the cells to 50%). The results are presented within the final report in the form of tabulated data, graph and photographs of the cell cultures before and after treatment.
- A range of acceptance criteria must be satisfied in order for the experimental run to be valid. These include compliance of control values within the ranges specified in our Standard Operating Procedure (SOP), and reproducibility of the 6 replicates with a CV% value ≤15%.

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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