TEST CODE:
CT-060

ISO 10993-5: Biological evaluation of medical devices – *in vitro* cytotoxicity

METABOLIC CAPACITY (MTT) OR MEMBRANE DAMAGE (NEUTRAL RED UPTAKE - NRU) METHOD

OVERVIEW

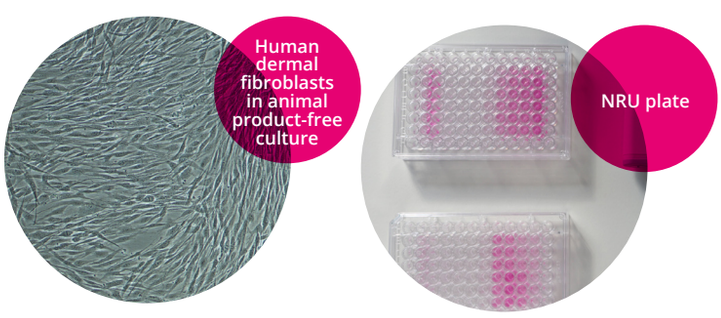
Cytotoxicity is defined as the degree to which a test item causes damage (toxicity) to cells. This may occur by one or more mechanisms including reduced metabolic capacity or damage to cell membranes.

The test described here is an extract method for the quantitative assessment of cytotoxicity either by metabolic capacity (MTT test) or membrane damage (Neutral Red Uptake - NRU). A sterile sample of the test item is incubated with a solvent for 24 hours, after which the solvent containing extracts from the sample is tested. 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%). Methods to determine qualitative cytotoxicity through direct or indirect contact are also available, performed through applying the test item directly to a cell layer, or to a layer of agar sitting on the cells surface. Although the qualitative direct and indirect contact methods are appropriate for screening purposes, it is commonly recognised that quantitative cytotoxicity is preferable.

The method utilises human dermal fibroblasts in animal product-free culture conditions. This test system provides the advantages of using a fully human cell based system to predict human toxicity, with maximal relevance to human physiology. The test item is prepared in cell culture medium. The test item is serially diluted to 12 test concentrations, and three 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% 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

TEST CODE:
CT-012

ISO 10993-5: Biological evaluation of medical devices – *in vitro* cytotoxicity

METABOLIC CAPACITY (MTT) OR MEMBRANE DAMAGE (NEUTRAL RED UPTAKE - NRU) METHOD

SUMMARY OF THE TEST METHOD

- If the test item is provided non-sterile, it is sterilised via a method approved by the client prior to set up.
- Human Dermal Fibroblast cells are seeded into a 96-well plate and cultured at 37°C / 5% CO2 under humidified conditions for 24 hours.
- For the extraction method, the test item, under sterile conditions, is submerged in solvent based on the extraction ratio (mass/volume). The samples are incubated at 37°C under agitated and circular motion for 24 hours.
- On the day of testing, medium is collected from the incubated sample and serially diluted in culture medium to give 12 test concentrations ranging from 100% to 10.77%.
- The negative control is Fibrolife medium and/or extraction medium.
- The positive control is dependent on the endpoint of the assay and can either be Triton X-100 (MTT method – assessing metabolic activity) or Sodium Dodecyl Sulphate (NRU method – assessing membrane integrity).
- For each test item, a control plate (consisting of 8 replicates) and a test item plate consisting of 3 replicates is set up.
- The dosed plates are incubated at 37°C / 5% CO2 for 48 hours for the MTT method or 24 hours for the NRU method.
- If 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.
- If 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. Any Neutral Red that has been taken up and retained by the cells is then solubilised by the addition of Neutral Red Solubilisation Solution, quantified by absorbance at 540nm using a spectrophotometer.
- Data is analysed to determine the EC50 value and the percentage viability values relative to untreated controls in order to determine the cytotoxic potential of the test item and the mechanism(s) involved.
- A range of acceptance criteria must be satisfied in order for the overall test to be considered valid.

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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