TEST CODE:
CT-067

Micronucleus Test

IN VITRO MAMMALIAN CELL METHOD (OECD TEST GUIDELINE 487)

OVERVIEW

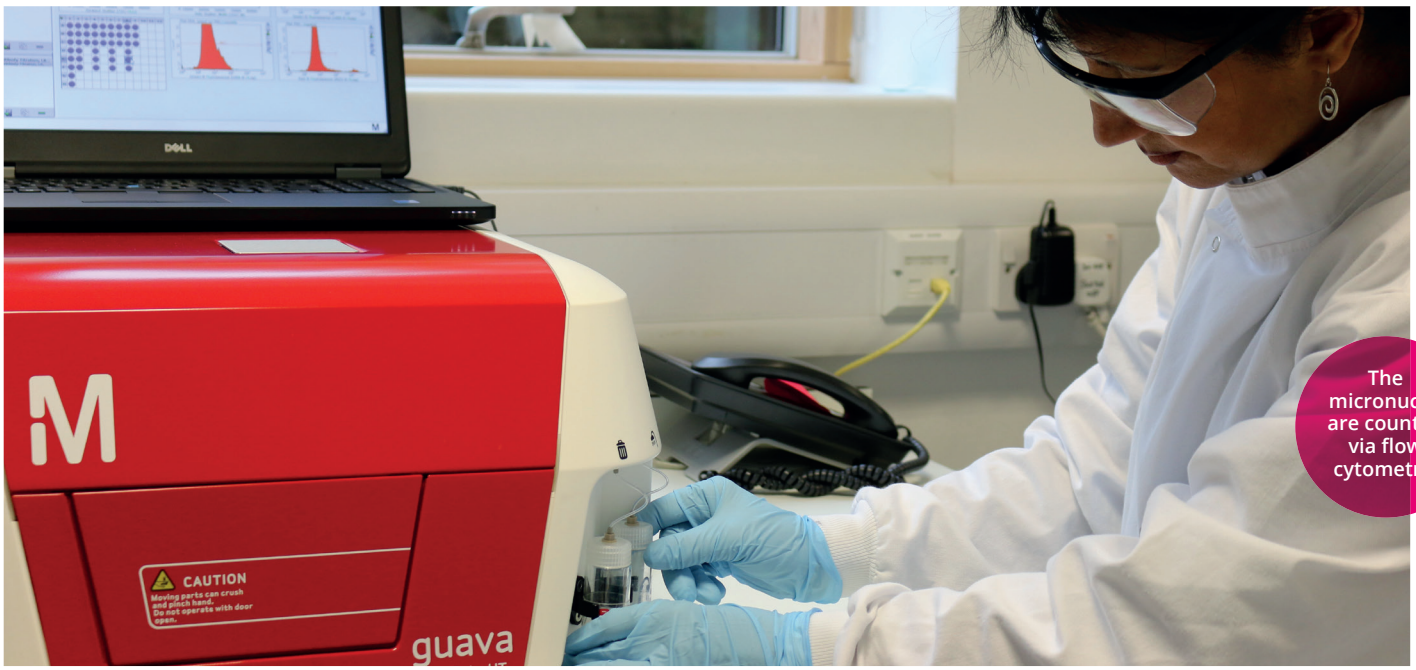
The *in vitro* Mammalian Micronucleus Test (MNvit) described here is a method to determine whether a substance is genotoxic. This is achieved through determining the number of micronuclei found in cells exposed to the test item. Micronuclei found in the cell cytoplasm are counted during the interphase of the cell cycle. This represents damage that has been passed on to daughter cells after cell division, meaning both aneugens (substances causing an abnormal number of chromosomes) and clastogens (substances which break or disrupt chromosomes) can be detected by the method.

Human TK6 cells are harvested in the exponential growth phase and incubated with the test item as several different concentrations for both short-term and long-term treatments (1.5 – 2 cell cycle lengths total). As the cells are not metabolically active, this is added to the test system using human S9 liver extract. Cell proliferation is measured to ensure the cells have divided during or following exposure to the test substance, and cytotoxicity to ensure sufficient viable cells are analysed.

The cells are lysed and stained with nucleic acid dyes before the micronuclei are counted via flow cytometry from a minimum of 2,000 cells per culture. A test item is determined as testing positive for the test and capable of causing chromosome loss/breaks if any of the following criteria are fulfilled: a statistically significant increase in the number of micronuclei are found in any of the experimental conditions compared to the controls; the increase in micronuclei is shown to be dose-related when analysed with a trend test; or the results are outside the distribution of historic negative controls.

TEST SYSTEM: HUMAN TK6 CELLS

TK6 cells are a suspension cell line of human lymphoblasts and are commonly used as the test system for micronucleus assays. The cells are maintained in the exponential growth phase in routine culture at 37°C / 5% CO2, while the culture medium and all culture reagents are free of animal-derived components.



The micronuclei are counted via flow cytometry.

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SUMMARY OF THE TEST METHOD

- Prior to the main study, the test item undergoes preliminary checks for solubility, precipitation, pH compatibility, and cytotoxicity.
- TK6 cells are seeded in a 96 well plate with 10 concentrations of the test item, determined by the solubility and cytotoxicity of the preliminary tests.
- Separate 96 well plates with cell and test item concentrations are prepared with and without metabolic activation using human S9 liver extract for a long exposure time, and without S9 for both long and short exposures. Different metabolic states and exposure times allow multiple controls to be used, providing control samples for both aneugens and clastogens. The cells are cultured without use of cytochalasin-B.
- In total, all cells are incubated on plates for 1.5-2 cell cycle lengths under standard culture conditions (37˚C, 5% CO2). Short exposure cells are washed after 3 hours, and incubated for the remainder of the 1.5-2 cell cycle lengths.
- At the end of the incubation period, the cells are washed, lysed, and exposed to a nucleic acid dye. Flow cytometry is used to acquire 5000 cells per culture condition, and micronucleus values determined. Cell counts are taken to calculate the relative increase in cell concentration or relative increase in nuclear counts.
- If a statistically significant increase in the number of micronuclei are found in any of the experimental conditions compared to the controls, the increase in micronuclei is shown to be dose-related when analysed with a trend test, or if the results are outside the distribution of historic negative controls, then the test item is determined as testing positive for being able to cause chromosome loss/breaks.
- When none of the above criteria are observed, the test it is determined as being negative, and unable to cause chromosome loss/breaks.

TURNAROUND TIME	*8-12 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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