TEST CODE:
CT-037

Genotoxicity Test

BLUESCREEN™ ANIMAL-FREE (BS-AF)

OVERVIEW

Genotoxicity can be defined as the ability of a test item to cause damage to the genetic material of cells, which may potentially lead to cancer. A genotoxic test item may be a mutagen (changing the DNA code), a clastogen (damaging or breaking the chromosomes) or an aneugen (changing the number of chromosomes). The term genotoxicity is often confused with mutagenicity, but as described above, mutagens are only one class of genotoxin. In order to gain insight into the potential of a test item to cause cancer, it is important to test for all three major mechanisms of genotoxicity.

The test described here is a non-regulatory method for the assessment of the genotoxic potential of test items. It provides classification as Genotoxic or Non-Genotoxic and captures all three types of genotoxin, detecting mutagens, clastogens and aneugens. The test does not identify which of the mechanisms is responsible for the specific genotoxic potential of the test item.

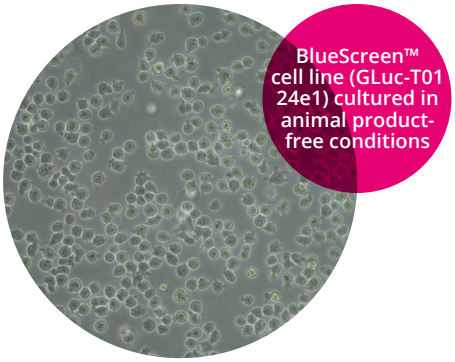
The human cells used in the BlueScreen™ test are derived from the TK6 human white blood cell line. The method detects changes in a stress pathway involving the GADD45a gene that is activated in human cells in the presence of genotoxins. The TK6 cells contain a specially inserted marker (luciferase) that converts an added substrate to emit light when the stress pathway becomes activated – this light signal is quantified using a luminometer and compared with controls to predict genotoxic potential. The test is performed both with and without human liver extract (S9) so that any genotoxic potential due to metabolism can also be predicted. Simultaneous assessment of cytotoxicity ensures that the results for genotoxicity are not adversely affected by basic toxicity to the human cell cultures.

The standard BlueScreen™ test included the use of animal-derived components in the test system, such as horse serum in the cell culture medium and rat liver extract (S9). In order to provide our clients with a truly animal-free test, XCellR8 has adapted the cell culture to animal product-free conditions, in collaboration with the developer and manufacturer of BlueScreen™, Gentronix Ltd. Horse serum has been replaced with human serum and rat S9 with human S9. Proficiency Testing demonstrated successful classification of a panel of genotoxic and non-genotoxic reference chemicals, with and without S9. A full technical report on the Proficiency Testing is available on request.

BlueScreen™ Animal-Free (BS-AF) is the first truly animal-free test for human genotoxicity, capable of predicting all major classes of genotoxin.

TEST SYSTEM: BLUESCREEN™ CELL LINE GLUC-T01 24E1

The BlueScreen™ cell line, known as GLuc-T01 24e1, is derived from the TK6 human white blood cell line. The cells are p53 competent and host a patented *Gaussia* luciferase (GLuc) reporter system which exploits the normal regulation of the GADD45a gene. Exposure to a genotoxic test item increases the expression of GADD45a (as part of a stress response) and hence *Gaussia* luciferase synthesis in the cells is also induced. The addition of a light producing luciferase substrate allows the signal to be quantified by luminescence detection.



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CT-037/02-05/18

XCellR8 Redefining testing

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SUMMARY OF THE TEST METHOD

- BlueScreen™ cells (GLuc-T01 24e1) are routinely cultured at 37°C / 5% CO_2 and seeded into 96-well plates for the test.
- Test items are prepared immediately prior to setting up the test, by serial dilution to 8 test concentrations. The final concentration of DMSO in the test plate wells is 1%.
- For each test item, 2 replicate wells are set up at each of the 8 concentrations.
- For each test item, 1 plate is set up with human liver extract (S9) and one without S9.
- The negative control is 1% DMSO and the positive control is aflatoxin B1 (with S9) or 4-nitroquinoline-1-oxide (without S9).
- The dosed plates are incubated at 37°C / 5% CO_2 for 48 hours.
- At the end of the exposure period, the plates are equilibrated to room temperature prior to the determination of luciferase activity (indicating genotoxic potential) and cell number (indicating cytotoxic potential): Plates are placed into a validated luminometer, which is programmed to add the luciferase substrate (50µl), shake for 2 seconds, then integrate luciferase activity. A combined preparation of cell lysis reagent and DNA binding stain (50µl) is added to each well and the plates are incubated for 20 minutes, protected from light. Cell number is then measured by fluorescence (485nm$_{ex}$ / 535nm$_{em}$) using a spectrophotometer.
- Data is analysed to determine whether any of the concentrations of test item exceeded the threshold for genotoxicity and / or cytotoxicity. If any of the test concentrations exceeded the defined luciferase threshold, the test item is classified as Genotoxic. If none of the concentrations exceeded the threshold, the test item is classified as Non-Genotoxic.
- A range of acceptance criteria must be satisfied in order for the test to be considered valid. These include a requirement to pass the required viability threshold in the parallel cytotoxicity test, ensuring that the data is derived from healthy, viable cells.

TURNAROUND TIME	*4 – 6 weeks*
AMOUNT OF SAMPLE REQUIRED	*10ml (liquids) / 10g (solids)*
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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