In vitro Genotox

Committed to developing alternative testing methods, JRF Global offers a customized battery of *in vitro* tests for both new ingredients and finished products.

In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene, Mouse lymphoma L5178Y TK+/- -3.7.2C cell line (OECD 490)

It is widely used as an alternate assay for checking gene mutation. The assay detects forward mutations in reporter gene the endogenous thymidine kinase gene. The thymidine kinase gene enables the detection of viable colonies, whose cells are deficient in the enzyme thymidine kinase following mutation from TK+/- to TK-/-. This deletion imparts resistance to trifluorothymidine (TFT) and can be selected for in a background of TK+/-. Mutant colonies have a bimodal size distribution, large and small colonies. The small colony detects gene mutations and large colony detects chromosomal events. This assay has an advantage, it can be detected both gene mutation like point mutation, frame shift mutation etc. and chromosomal event like large deletion, chromosomal rearrangement and mitotic recombination.

Bacterial Reverse Mutation Test (Ames test) using Salmonella typhimurium and E. coli WP2uvrA (OECD 471)

It detects mutations which revert mutations originally present in the tester strains and which restore the functional capability of the bacteria to synthesise an essential amino acid. The revertant bacteria are detected by their ability to grow in the absence of the amino acid required by the parent tester strain. The tester strains used for the bacterial reverse mutation test are the histidine auxotrophic strains of Salmonella typhimurium TA98 and TA1537 (Frame shift mutation), TA100, TA102 and TA1535 (Base pair substitution) and tryptophan auxotrophic strain of Escherichia coli WP2uvrA (Base pair substitution). The bacterial reverse mutation test is rapid, inexpensive and relatively easy to perform. JRF has expertise and huge historical data base to conduct study by using different methods and customize protocol based on chemical properties of the test item.

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In vitro Mammalian Chromosome Aberration Test using Human Peripheral Blood Lymphocytes or Chinese Hamster Ovary - K1 Cell Line (OECD 473)

The *in vitro* chromosomal aberration (CA) test detects structural aberrations and may give an indication for numerical chromosome aberrations (polyploidy) in cultured mammalian cells caused by the test chemical. At JRF, we conduct *in vitro* chromosomal aberration test using either CHO-K1 cell line or human peripheral blood lymphocytes. Cytotoxicity is evaluated by assessing inhibition of mitotic index. Cells in metaphase are analysed for the presence of chromosomal aberrations both in the absence and presence of S9 activations. JRF has adopted all the revised OECD guideline changes (OECD, 2014).

In vitro Mammalian Cell Gene Mutation Test (HPRT assay) using Chinese Hamster Ovary-K1 cell line (OECD 476)

Cells deficient in Hypoxanthine-guanine Phosphoribosyl Transferase (HGPRT or HPRT), due to mutation, are resistant to the cytotoxic effects of the purine analogue (6-thioguanine). HPRT proficient cells are sensitive to 6-thioguanine which causes the inhibition of cellular metabolism and halts further cell division.

The assay can detect a wide range of chemicals capable of causing DNA damage that leads to gene mutation. The test follows a very similar methodology to the thymidine kinase (TK) mouse lymphoma assay (MLA), and both are included in the guidelines for mammalian gene mutation tests (OECD (1997). The HPRT methodology is such that mutations which destroy the functionality of the HPRT gene are detected by positive selection using a toxic analogue, and HPRT –mutants are seen as viable colonies. JRF has conducted more than 100 GLP studies as per different regulatory guidelines such as OECD, OCSPP (EPA) and EC.

In vitro Micronucleus Test Using Human Peripheral Blood Lymphocytes and CHO-K1 cell line (OECD 487)

JRF Global's top-notch genotox team is proud to offer the *in vitro* micronucleus test as a preferred alternative to the *in vitro* chromosomal aberration test.

Genotoxic carcinogenic compounds change normal cell behavior by damaging DNA. One of the mechanisms for DNA damage is attributed to incorrect chromosomal replication. During normal cell division, an equal number of replicated chromosomes are present in the nuclei of both daughter cells. In the presence of certain carcinogenic compounds, however, the replication process is disrupted and a piece of the replicated chromosome breaks away and is not incorporated in either of the daughter nuclei. Such a chromosome may then go on to form a micronucleus.