



XENOMETRIX
by Endotell

Genotoxicity Testing Ames MPF™ Fluctuation Assays
Cytotoxicity Screening IN CYTOX Cytotoxicity Test Systems

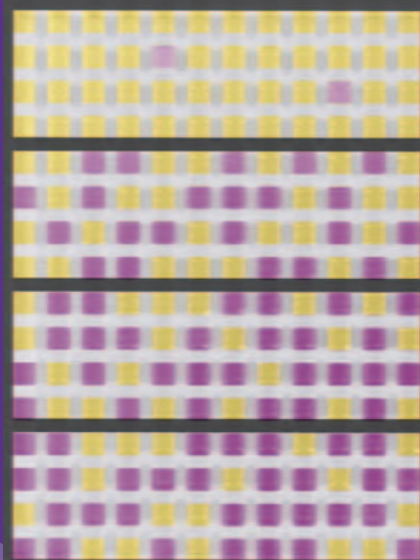
Genotoxicity Testing from Xenometrix

Ames MPF™ Fluctuation Assays

All new compounds are tested for carcinogenic properties during the development process and are required to be tested for genotoxicity potential as part of the regulatory submission. The most common test for genotoxicity is the Ames plate incorporation test developed in the early 1970's by Bruce Ames and his team at the University of California, Berkeley, USA and which forms the basis of the OECD Guideline 471 for the in vitro testing of chemicals.

However with the increasing demand for new chemicals, shorter development times and lower costs the traditional Ames plate incorporation test is not ideal as it is labour intensive, expensive, more sensitive to errors, produces more waste for disposal and uses at least 3 times more test compound.

To overcome the drawbacks of the original Ames test, Xenometrix have developed the Ames MPF™ Fluctuation Assay which is an "off the shelf" package and which provides a far more convenient standardised system of analysis with significantly reduced costs per test, minimised operator intervention plus greater confidence in the results.



The benefits are:-

- 10 times lower cost per analysis
- 250 times less contaminated waste
- 3 times less compound required
- 5 times less operator intervention
- Ready-to-use reagents and quality controlled bacterial strains
- No preliminary autoclaving or sterility testing required
- Colorimetric determination to reduce reporting errors
- OECD Guideline 471 and FDA compliant

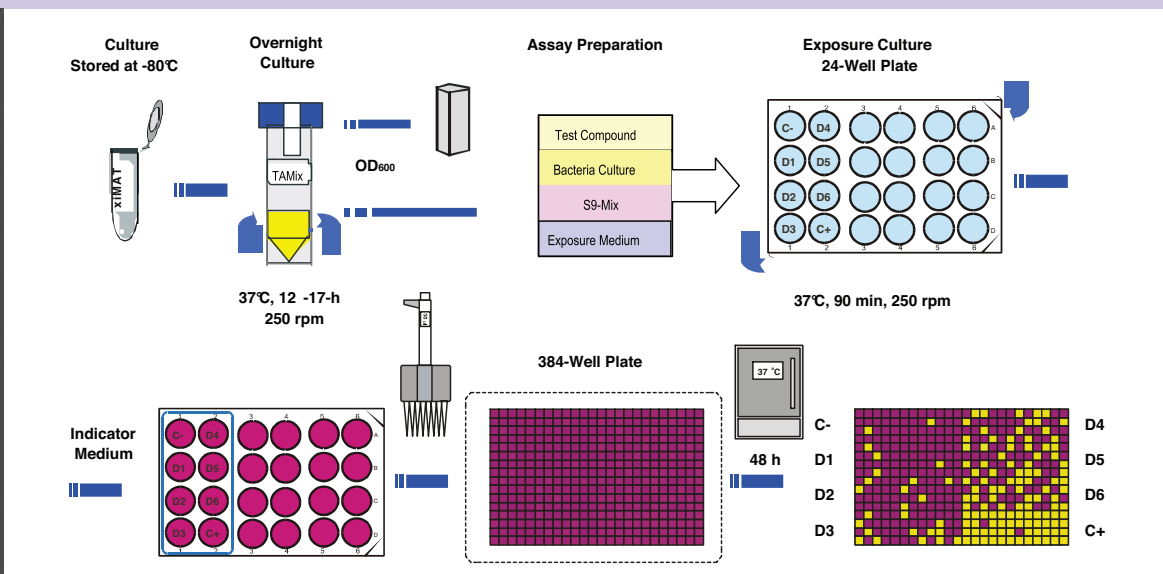
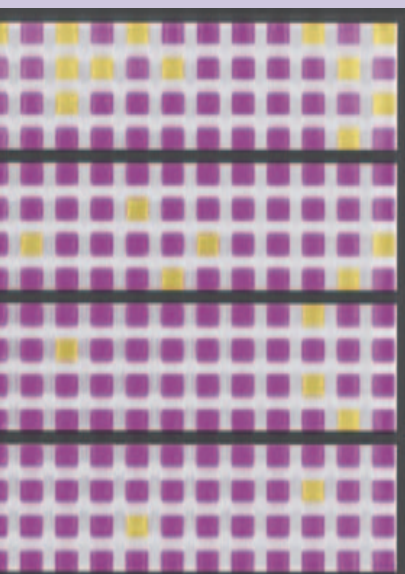
Principles

The Ames test is a reverse mutation test using several strains of *Salmonella typhimurium* which carry mutations in the genes for the synthesis of the amino acid histidine. This means that they require histidine for growth. When the bacteria are exposed to mutagenic agents this results in a reverse mutation of the genes and the bacteria will now grow in a histidine-free medium to give a positive result. The *Salmonella* strains are selected to investigate indicative base-pair and frameshift mutations plus they may be used with the rat liver extract S9 which stimulates metabolism for the study of metabolic product effects.

The bacteria are exposed to six different concentrations of the test chemical as well

as a positive and negative control for 90 minutes in a medium containing sufficient histidine to support approximately two cell divisions. After 90 minutes the exposed cultures are diluted with a pH indicator medium lacking histidine and aliquoted into 48 wells of a 384 well microplate. Within two days cells which have mutated will grow into colonies and their metabolism will lower the pH of the medium resulting in a colour change from purple to yellow. The colour change is easily detected by eye or may be more formally documented by use of a microplate reader. A greater than two-fold increase and a dose related increase in the number of mutant colonies following exposure to the test chemical relative to the zero-dose control indicates that the chemical is mutagenic and will require further investigation.

Faster analysis reduced costs and convenience – what more do you need? A new approach to a traditional problem



Test Kits

The Ames MPF™ Fluctuation assay test kits are available in different combinations to suit individual requirements .

(please see ordering guide for further information)

Single strain kits TA98, TA100, TA1535
TA1537 & E.coli WP2

2-strain kits TA98 & TA100, TA98 & TAMix

4-strain kits TA98, TA100, TA1535 & TA1537
5- strain kits TA98,TA100,TA1535 TA 1537 & E.coli WP2

The test kit includes all the necessary components in a ready-to-use form to analyse at least 10 samples in triplicate or 30 samples without replicates, at six different concentrations with positive controls and with or without S9 metabolic activation. Clear, step-by-step instructions for use are

also included. Kits for the testing of single compounds are available or you may design your own kit as all materials are sold separately.

For laboratories who are presently using the traditional Ames plate incorporation test, the quality controlled Salmonella strains (phenotyped and genotyped) will avoid the requirement for phenotype testing and the positive controls will be beneficial for data integrity.

The tests may be performed in either 384 or 96 well microtitre plates and therefore are perfect for laboratories who have a microplate reader and who require higher levels of automation or analysis which is independent of operator observation.

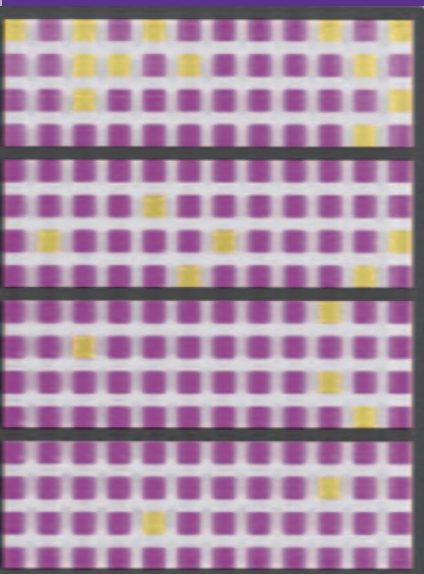
Who will benefit

The Ames MPF™ Fluctuation Assay may be used to detect genotoxic activity in chemicals, pharmaceuticals, foodstuffs, cosmetics, environmental and many other sample types. Any laboratory who is involved in new product development, toxicology or who is covered by the REACH directive from education to industry or government are sure to benefit from this new, convenient, reliable and cost effective approach to genotoxicity testing.

The Ames MPF™ test kits have been evaluated by several major pharmaceutical and regulatory organisations and are now in routine use in their laboratories.

Cytotoxicity Screening From Xenometrix

IN CYTOX Cytotoxicity Test Systems



The requirement to evaluate the potential toxicity of compounds used in a wide range of products from pharmaceuticals, cosmetics, food additives and pesticides to industrial chemicals or fertilisers is growing rapidly due to commercial pressures and increased regulation. The test methods need to be reliable, sensitive and suitable for increased productivity with multiple testing on the same sample.

Cytotoxicity assays were one of the first in vitro tests to predict the toxicity of substances to various tissues and are now widely used for the determination of cell proliferation, viability or structural activity. The added benefit of such assays is that they limit the requirement for animal experimentation and the screening requires much smaller quantities of the test material.

Xenometrix have developed a convenient range of products for the in vitro evaluation of tolerance, resistance and recovery of cells in response to chemical interactions.

The benefits are:-

- Complete range of Cytotoxicity assays
- Flexible combination of assays
- Fast and efficient analysis
- Convenient operation
- Single or multiple assays on one sample
- Reduced amount of test material when using multiple parameter assays
- Direct comparison of results with multiple parameter assays
- Several cytotoxic mechanisms may be analysed on the same sample
- Lower cost per analysis
- Reliable performance due to quality controlled reagents and detailed instruction manual

Principles

The Xenometrix Cytotoxicity assays consist of four steps:

Trypsinisation of the cells

Transfer to 96 well microtiter plates

Test compound incubation

Cytotoxicity assay

The cells are harvested by trypsinisation, counted, diluted and transferred to 96 well microtiter plates. Following incubation for 24 hours dilutions of the test compound are added, the cells exposed for several hours and then the cell growth and viability are tested using one or several of the cytotoxicity assays. The assays are measured using a microplate reader with protocols specific to the assay.



Test Kits

Individual assay kits, including all the reagents necessary to perform the assay, are available for membrane integrity (LDHe), metabolic activity (GLU), respiratory chain activity (XTT/MTT), total protein synthesis (SRB), DNA cell content number (CVDE) and lysosomal activity (PAC,NR). The assays will determine the 50% inhibiting dose or concentration (ID50 or IC50) no effect dose (NED) and inhibiting time (IT50). (please see ordering guide for further information)

The XTT assay for respiratory chain (mitochondrial) activity is especially beneficial

as the product is water soluble and therefore does not require an overnight solubilisation step plus it is at least twice as sensitive as the MTT assay. The LDHe assay is also unique in that it is insensitive to pyruvate in the culture medium unlike other assays hence ensuring higher quality information.

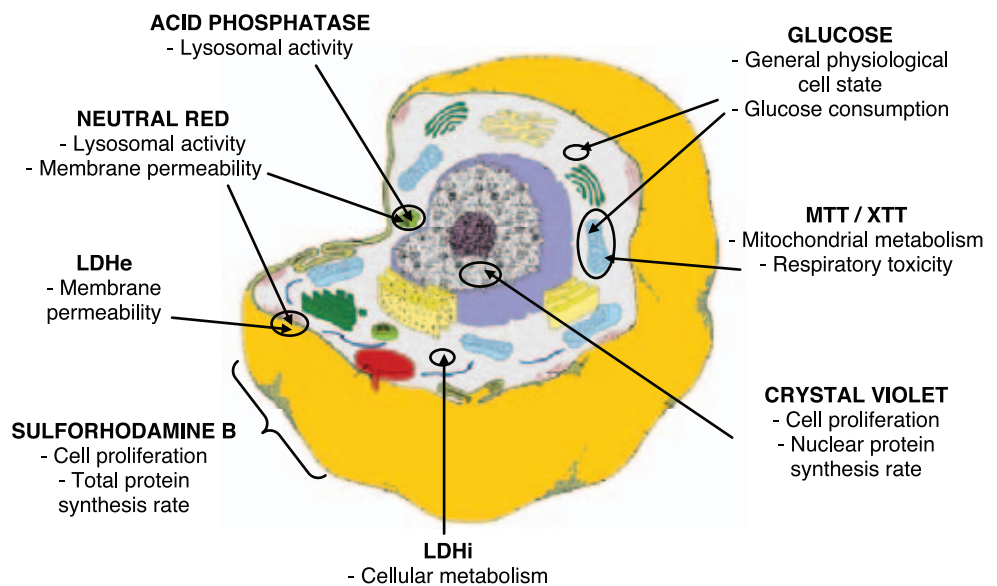
It is also possible to combine up to four assays in one test on the same cellular sample. For example membrane integrity, cellular metabolism, mitochondrial activity and total protein synthesis assays may be performed on the same sample. The combined assays enhance the relevance of the results, reduce sample handling and

minimise the amounts of test compound required. This is especially important when using valuable samples such as primary cell cultures.

CelTox Software

CelTox software has been specifically developed for data handling and reporting of the various assays. The software includes information on the cell lines used, culture conditions, test compounds and assays performed together with ID50 or IC50 values and graphical presentation of the results. The CelTox software is available separately.

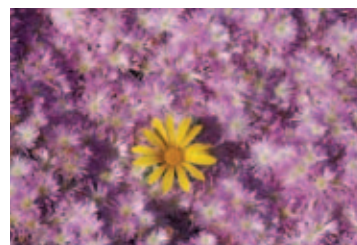
Cytotoxicity Test Systems



Optimised Assays from Xenometrix - Experience the difference

Environmental Samples

Many laboratories are now actively involved in testing air, soil and water for genotoxicity and cytotoxicity, which are quite demanding types of analysis. Xenometrix test kits are ideally suited to high productivity and for the standardisation of methods required for testing waste, river, or drinking water, soil, air or other effluents. Xenometrix have a comprehensive knowledge of these sample types and are able to provide technical support to assist laboratories in establishing the analyses as standard techniques.



Support and Training

Xenometrix pride themselves on their customer support and provide assay optimisation advice, regulatory information, kit customisation, publications, plus the customer help-lines are operated by experienced scientists so that enquiries are handled as efficiently as possible.

Training is available either in the Xenometrix, Basle, facility or on site if required with certificates of competence being issued upon successful completion of the training.



Client Contract Laboratory

The Xenometrix facility in Basle has a fully staffed and equipped laboratory for the purpose of performing optimal Ames testing on customer samples. Xenometrix performs the assays, interprets the data and then produces a detailed analytical report which is a cost effective alternative for customers who do not have the resources to perform the tests themselves or who are interested in confirmation analysis.



Specialist Resources

A wide range of individual items are available for the independent research chemist who prefers the more traditional methods of testing or developing their own process. These items include incubation media, quality controlled Salmonella strains, rat liver S9 etc, plus all associated plastic ware.

Whatever the question regarding AMES testing or Cytotoxicity screening, Xenometrix have the answers



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