NEW

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GLP AMES

GLP OECD Guideline 471 Ames bacterial reverse mutation screening assay

A bacterial reverse mutation test (often simply referred to as an 'Ames test') is a core component of safety assessment testing batteries performed in amino acid-requiring strains of *Salmonella typhimurium* or *Escherichia coli*. Point mutations, involving substitution, addition or deletion of one or a few DNA base pairs, are detected when they cause restoration of the functional capability of the bacteria to synthesize an essential amino acid. Hence, revertant bacteria are able to grow in the absence of the amino acid required by the test strain.

This assay uses the traditional bacterial reverse mutation test described in OECD Guideline 471. It is based upon scoring bacterial growth (colonies) on selective agar plates after exposure of the bacterial cells to a test item, either by incorporation of the test item into the agar plates, or by pre-incubation prior to plating out.

Strains

- *S. typhimurium* TA98
- *S. typhimurium* TA100
- *S. typhimurium* TA1535
- *S. typhimurium* TA1537
- Either:
  - *E. coli* WP2 *uvr*A
  - *E. coli* WP2 *uvr*A pKM101 or
  - *S. typhimurium* TA102

Number of dose levels

5 analysable dose levels will be carried out.

Number of replicates

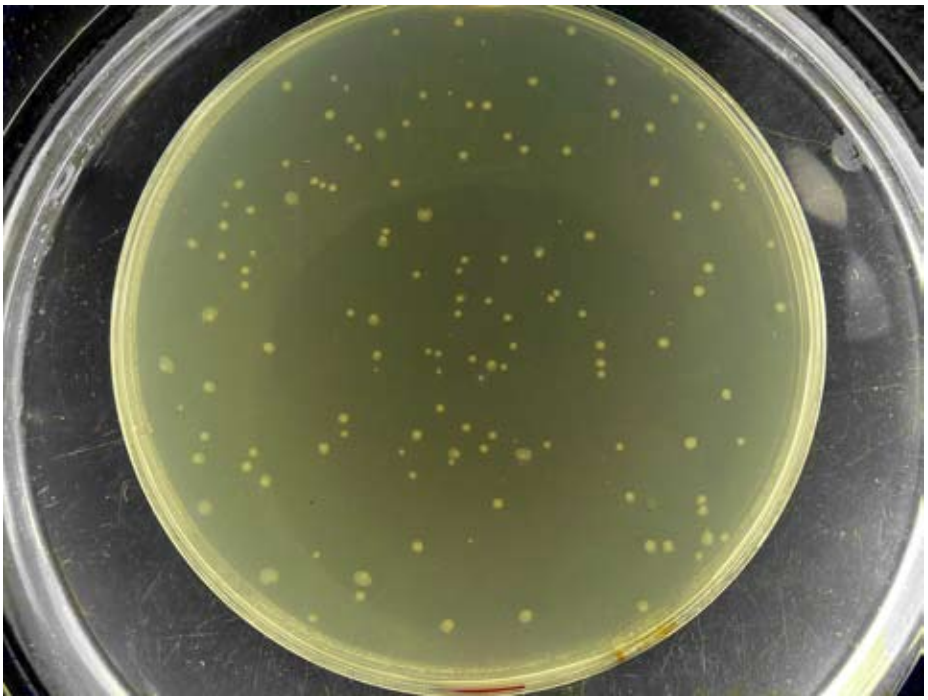
3 replicates will be carried out.

Test item requirement

1.5g of test item will be required (includes range-finder testing and formulation analysis).

Turnaround time

Turnaround time from receipt of test article and characterisation/ formulation information, to submission of the final report for sponsor approval is typically 50 days..



Process

When requesting a GLP study, the sponsor will typically provide test item characterisation, formulation information, and analytical methods for formulation analysis.

A draft study plan is prepared for review by the sponsor, Gentronix, and an independent QA group. Once this is approved and the test items supplied, the study can begin.

If no toxicity or solubility information has been provided, a range-finder test is carried out before the main assay is performed. If formulation analysis is required, this is carried out immediately after dosing.

The sponsor is notified of the results of the study.

A draft report is prepared and reviewed by the sponsor, Gentronix and the QA group. Once approved, a QA statement is added before the final report is issued.

Assay Principles

Culture Growth

Each test strain is incubated at 37°C, with shaking, for 8-10 hours to achieve a density of 1-2x10^9 bacterial cells per ml.

Dosing

Test item is assayed across typically 5 analysable dose levels with solvent and positive controls, all performed in triplicate. The test is operated both with and without exogenous metabolism (S9 mix).

Pre-incubation

In some cases, for example if the test item is a volatile liquid, it is pre-incubated in a sealed vessel for 1 hour at 37°C, with shaking.

Plating out

Either after pre-incubation or directly for solid test items, the bacterial suspensions, test items and top agar (trace histidine/tryptophan) are mixed and poured onto the agar plate.

Incubation

Plates are inverted and incubated at 37°C for 3 days.

Scoring

The numbers of revertant colonies are counted, either manually or using an automatic plate counter.

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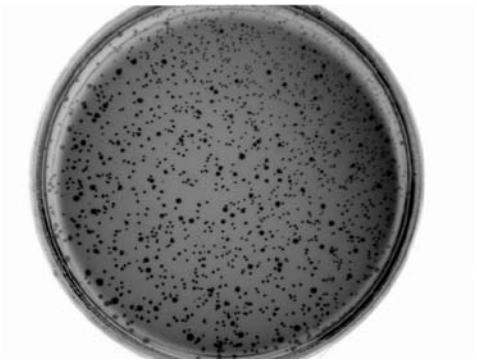
innovation and service

Gentronix is an established biotechnology innovation and service company specialising in early screening, mechanistic follow-up and regulatory genotoxicity assays for a range of industries including; pharmaceuticals, chemicals, agrochemicals, personal care, consumer products, flavours, fragrances and taste enhancers, and medical devices.

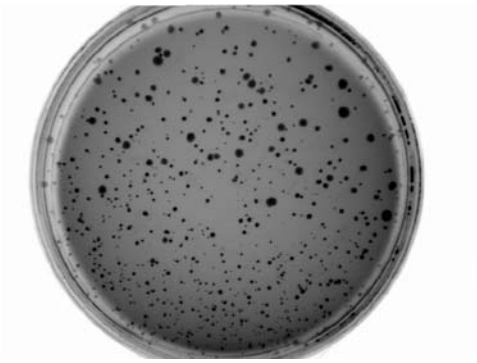
In addition to classical genotoxicity screening assays, Gentronix offers GreenScreen®HC and BlueScreen™HC which are novel, patented systems that, unlike earlier assays, detect all known classes of genotoxin.

Gentronix can provide assays and advice on follow-up strategies for positive results, and mechanism elucidation to help chemists modify compounds to eliminate genotoxicity early in product discovery and development thereby preventing late stage failure.

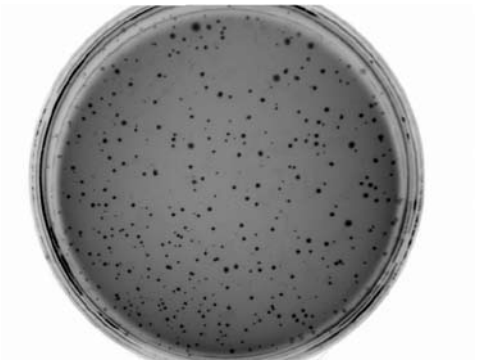
Gentronix is GLP compliant and offers regulatory assays to OECD and other test guidelines.



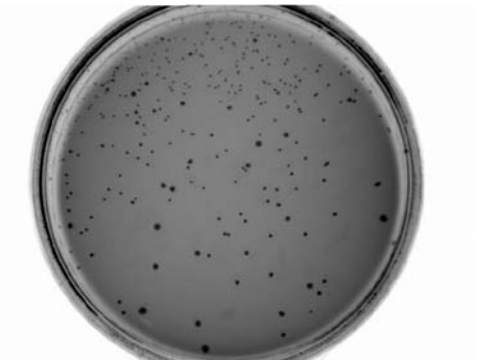
TA98 (+S9) + 2 µg/plate 2-aminoanthracene



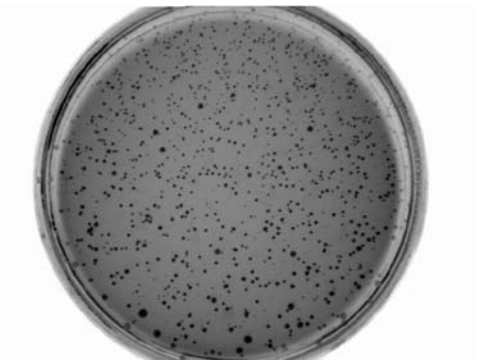
TA100 (-S9) + 0.5 µg/plate sodium azide



TA1535 (-S9) + 0.5 µg/plate sodium azide



TA1537 (+S9) + 2µg/plate 2-aminoanthracene



E. coli *(-S9) + 25 µg/plate potassium dichromate*

A selection of positive control plates read using a Sorcerer Petriviewer Mk 2 and software.

***AM-FL03** V1, Aug 2014 © Gentronix Ltd*

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