BioReliance

Toxicology Services

# Ames II and GreenScreen HC assays

BioReliance is pleased to offer an expanded set of genetic toxicology screening assays. The addition of Ames II and GreenScreen HC provides our clients the opportunity to test for genetic toxicity earlier in the development process and with much less test article required for each assay. The highly predictive nature of these assays makes them ideal for screening during lead optimization.

The standard ICH battery of genetic toxicology assays has been performed just prior to initiation of clinical trials for many years. While a positive result in these assays does not immediately halt development of that compound, knowing which compounds would eventually test positive could certainly change the priority within a pre-clinical pipeline. The combination of the Ames II and GreenScreen HC assays has been shown to be highly predictive of both the standard Ames assay, but more importantly the combination is predictive of the other ICH assays – *in vitro* chromosomal aberration, mouse lymphoma, and micronucleus assay. The combination of Ames II and GreenScreen HC provides for a complementary test battery with high sensitivity (detection of genotoxic compounds) and high specificity (avoiding false positives).

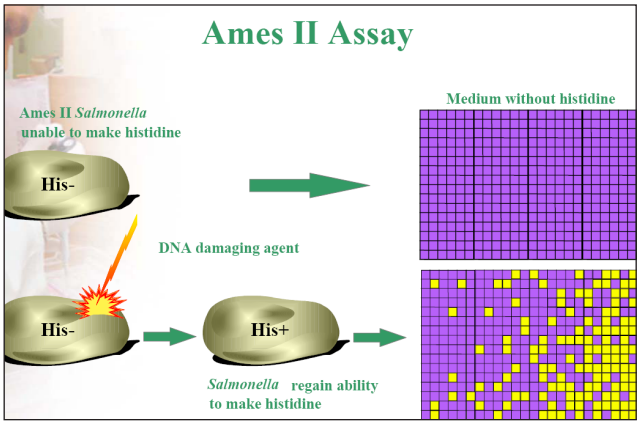
Today the ICH test battery requires grams of test article, and even the scaled-down versions require hundreds of milligrams of test article. The introduction of these screening assays that require 1–10 milligrams of test article each, will allow compounds to be tested earlier, when the amount available from the chemistry labs is very limited.

Additionally, the higher-throughput nature of these assays decreases significantly the amount of time needed to get results. In less than two weeks from test article submission you will have written results of these assays, speeding your development efforts.

## Ames II assay

The Ames II assay is a second generation bacterial reverse mutation assay developed as a predictive screening assay for genotoxicity. The assay, a modification of the traditional "Ames Assay", was developed in Dr. Bruce Ames' laboratory at the University of California and commercialized by Xenometrix. The traditional Ames assay has been modified to permit the assay to be used as a high-throughput screening assay that requires very small amounts of test article. The assay is based on the detection of the presence or absence of mutants in each of many microwells as measured by cell growth.

The Ames II assay can detect both frameshift and base-pair substitutions under both nonactivation and exogenous (S9) metabolic activation conditions. Frameshift mutations are detected using the traditional TA98 Salmonella strain. The different types of base-pair substitutions are detected utilizing six new *Salmonella typhimurium* strains specifically engineered for the assay. Each strain carries a different missense mutation in the histidine operon that is designed to revert uniquely to one of the six possible base substitution combinations causing transitions or transversions in base sequence. The six strains are combined into a single culture called TAMix. The TAMix strains have a lower spontaneous reversion frequency compared to the standard *Salmonella* tester strains which makes it easier to utilize a microplate fluctuation format. A validation study performed by Xenometrix a few years ago, indicated the concordance of the assay was 87% with the standard *Salmonella* strains[1].




## The advantages of the Ames II system include:

- Ames II TAMix strains can be combined and used as a single mixture for rapid screening for base-pair substitutions
- Combining TAMix with TA98 permit effective screening for both basepair and frameshift mutagens with only two cell cultures
- Lower spontaneous reversion frequencies allow detection of mutagens at lower concentrations without loss of sensitivity
- Liquid format in microtiter plates leads to increased sensitivity and ease of automation

Originally licensed to Xenometrix, BioReliance has now licensed this technology on a worldwide basis. At the same time, BioReliance has now become the official distribution point for the original Ames bacterial strains from Bruce Ames.

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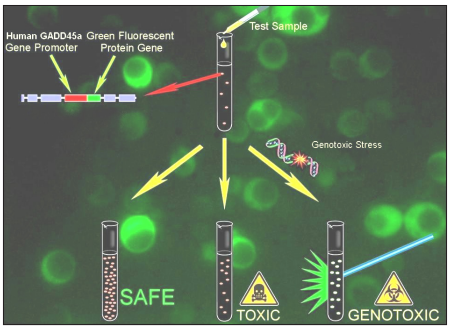
## GreenScreen HC assay with and without S9

The GreenScreen HC assay was developed by Gentronix and has been licensed by BioReliance. This human cell-based genotoxicity screening assay links the regulation of the human GADD45a (Growth Arrest and DNA Damage) gene to the production of Green Fluorescent Protein (GFP) (2). GADD45a is a gene that is central in the cellular response to all types of DNA damage. Cells that have incurred DNA damage express higher levels of detectable Green Fluorescent Protein. Hence when the test article causes DNA damage in the cell, the level of fluorescence induction increases. This assay uses the human p53-competent cell line TK6 as host for a reporter which includes the promoter region and regulatory gene sequences of human GADD45a gene operatively linked to a human codon-optimized GFP gene. Control cells carry the same reporter but the GFP gene is not expressed. The GFP carrying cell detects genotoxicity while the non-GFP cell allows data correction for compounds which themselves fluoresce. Simultaneous measurement of optical density in the assay allows normalization of fluorescence data as well as estimation of relative suspension growth, one of the ICCVAM recommended measurement endpoints for basal cytotoxicity. The major advantage of this assay is the lack of positive response to treatment conditions in other in vitro genetic toxicology assays that lead to artifactual (false) positives. The GreenScreen HC assay has been developed as a microplate or flow cytometric assay, and requires very little test article, on the order of 1 mg for testing up to 1mg/ml.




### The advantages of the GreenScreen HC system include:

- Very low amounts of starting material
- Microplate format provides for high-throughput (easily 40 compounds per day manually) and ability to automate
- Picks up multiple classes of genotoxicity including aneugens
- Provides both high specificity and high sensitivity
- Provides both nonactivation and S9 metabolic activation conditions

## High sensitivity without loss of specificity*

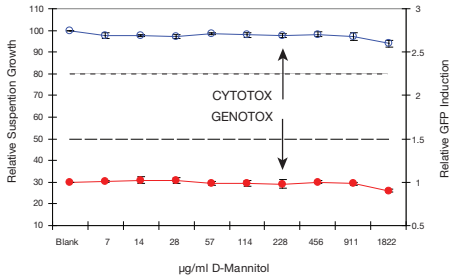
| | Concordance | Sensitivity | Specificity |
|---|---|---|---|
| Ames | 92% | 71% | 100% |
| MLA | 88% | 100% | 78% |
| In vitro CA/MNT | 79% | 100% | 63% |
| In vivo CA/MNT | 89% | 95% | 93% |
| **GreenScreen HC** | **98%** | **95%** | **100%** |

* Data above is taken from reference 2, and is relative to genotoxic carcinogenicity data.

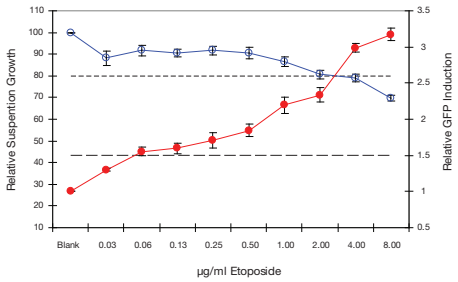
Data from GreenScreen HC assay could be useful in identifying those compounds with genotoxicity liability as early as hit to lead:

- **Positive** GreenScreen HC = likely **positive** *in vivo* and **positive** *in vitro* mammalian cells.
- **Negative** GreenScreen HC = likely **negative** *in vivo* and **negative** or a "false positive" *in vitro* mammalian cells.

Example of non-genotoxic and non-cytotoxic chemical (D-mannitol)




Example of genotoxic and cytotoxic chemical (etoposide)




Blue line and left Y axis show relative suspension growth as measure of cytotoxicity.Reduction to 80% of control reflects a full cell doubling but is a statistically significant toxic effect.
Red line and right Y axis show relative GFP induction as measure of genotoxicity. Increase of 1.5 over control is considered genotoxic.

## References


[1] **Gee P, et. al.** Comparison of responses of base-specific *Salmonella* tester strains with the traditional strains for identifying mutagens: The results of a validation study. Mutation Research, 1998; 412: 115 – 130.

[2] **Hastwell PW, et. al.** High-specificity and high-sensitivity genotoxicity assessment in a human cell line: Validation of the GreenScreen HC GADD45a-GFP genotoxicity assay. Mutation Research / Genetic Toxicology and Environmental Mutagenesis 2006; 607:160-175.

[3] **Jagger, C, et. al.** Assessment of the genotoxicity of S9-generated metabolites using the GreenScreen HC *GADD45a-GFP* assay, Mutagenesis, pp.1–16, September 2008.



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