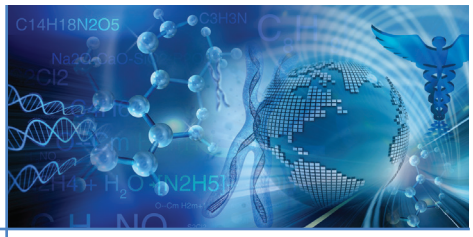
BioReliance

Toxicology Services



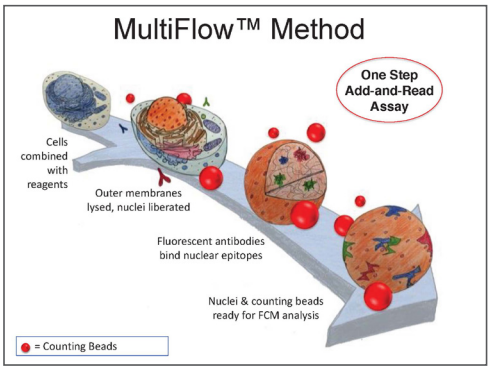
CAN MultiFlow™

Screening assay to determine Mode of Action of Genotoxicants

BioReliance has qualified and fully validated an assay that uses TK6 cells to **screen** for **C**lastogens, **A**neugens and **N**on-Genotoxicants using **Flow** Cytometry. All types of substances, compounds and product formulations can be tested for the presence of genotoxic modes of action that may yield positive findings and inhibit entry into further product development.

What is CAN MultiFlow™

CAN MultiFlow is a high-throughput 96-well assay using human TK6 cells. BioReliance has validated a proprietary method and design using Litron's MultiFlow DNA Damage Kit. The assay uses a logistical regression model to predict the clastogenic, aneugenic, or non-genotoxic properties of test articles based on test article-induced changes of: p53, γH2AX, Phospho-Histone H3, and polyploidy. Test articles can be loaded on to plates in multiple formats allowing for customization and optimization to fit specific needs and specific compounds. Flow cytometric analysis is employed allowing for high-throughput detection.



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Screening and Genotoxicity

Toxicity assessment and specifically Genotoxicity testing is an important aspect of product development. All industries and regulatory bodies require some sort of safety and hazard assessment of all types of substances, ingredients, or drugs. Acceptance of a product prior to first in human studies is determined using GLP Genotoxicity assays. Genotoxicity (DNA damage) can occur in a number of different ways, including mutation, clastogenicity and aneugenicity, The purpose of screening is to assess the potential Genotoxicity of compounds before performing GLP assays. Although GLP assays are not expensive within the overall costs of product development, screening assays are less expensive, have faster turnaround times, require less test article. Therefore they are more efficient in predicting the success or failure of a compound. Traditionally, non-GLP Genotoxicity screening assays are intended to predict results in GLP assays, and are less focused on determining Mode of Action (MOA).

MultiFlow™ Reagents

REAGENT	PURPOSE
Anti-phospho-H3-PE	Metaphase cell marker
Anti- γH2AX-Alexa 647	DNA double strand break marker
Anti-p53-FITC (human only)	Genotoxicity marker

Fully Qualified and Validated Commercial Assay

More Efficient, More Information, in Less Time

- Faster high-throughput screening
- Better information for compound development
- Improved fit for multi-phasic compound development

Applicable for All Industries and Compound Types

- Pharmaceuticals
- Industrial chemicals
- Agricultural chemicals
- Flavors and fragrances
- Consumer products

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BioReliance

Toxicology Services

CAN MultiFlow™

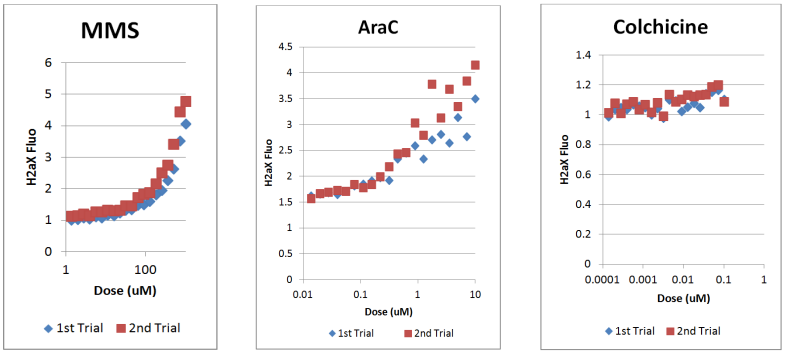
Uses and Benefits of CAN MultiFlow™

This assay is used to predict genotoxic as well as MOA properties of test articles, which cannot be done in other screening assays. Moreover this assay provides concurrent analysis of multiple biomarkers (p53, γH2AX, Phospho-Histone H3) and thus avoids the limitations of a single assay or biomarker. A test compound can be classified as a clastogen, aneugen, or non-genotoxicant. Identification of a clastogen means that the compound is DNA reactive with a linear dose response and only very low or no safe exposure is possible; and thus problematic for product development. On the other hand, an aneugen is a non-DNA reactive genotoxin that may have a threshold response and a margin of safety may be identified that allows for the compound to continue in development. The third classification is non-genotoxicant which could therefore be safe for further development. The high through-put design allows short turn-around times using minimal amounts of test article. The MOA information can also be used for designing GLP assays in a smarter way to avoid costly and time consuming follow-up assays.

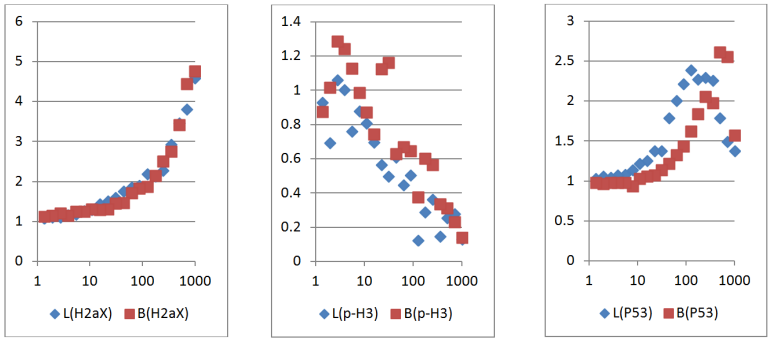
Validation Data

S9-Activated		Non-Activated			
Chemicals	**Model Predicted**	**Chemicals**	**Model Predicted**	**Chemicals**	**Model Predicted**
BaP	Clastogenic	MMS	Clastogenic	CCCP	Toxicant
CP	Clastogenic	Arac	Clastogenic	Pyrene	Non-genotox agent
Thapsigargin	Toxicant	Vinblastine	Aneugenic	Thapsigargin	Toxicant
Tunicamycin	Toxicant	Colchicine	Aneugenic	Tunicamycin	Toxicant

Assay Reproducibility



Assay Inter-Laboratory Comparison



For more information please consult information on specific assays, BioReliance's website or a BioReliance representative.

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