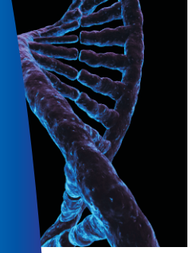
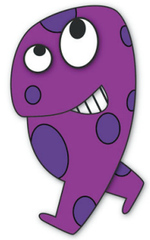
environmental
bio-detection
products inc.

6800 Campobello Rd
Mississauga, Ont, Canada
905.487.7359 ph
905.794.2338 fax
www.Biotoxicity.com

Measuring the **Health**
of the Environment

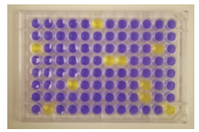


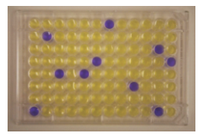
MUTA-CHROMOPLATE™



THE PRINCIPLES OF THE MUTA-CHROMOPLATE Reverse Mutation 'Ames Test'

The **Muta-ChromoPlate** is a 96-well microplate version of the Salmonella *typhimurium* 'Ames Test,' used for detection of mutagenic activity.
The **Muta-ChromoPlate** provides a clear colour endpoint. Reagents, cultures and other consumable components are supplied ready-to-use in a non-specialized laboratory.

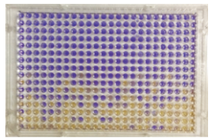




AMES - 384 ISO™
384 Well Format

THE PRINCIPLES OF THE Ames-384 ISO Reverse Mutation 'Ames Test'

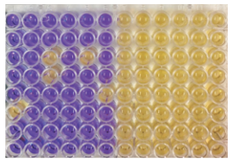
EBPI's **Ames ISO** procedure was developed to provide the same reliable assessments of mutagenic activity in environmental samples as the Muta-Chromoplate with the added benefits of using smaller sample sizes, less reagents and plastics, a pre-exposure period and less waste.



AMES - MOD ISO™
96 Well Format

THE PRINCIPLES OF THE AMES-MOD ISO Reverse Mutation 'Ames Test'

This method combines the benefits of the ISO method while maintaining the ease-of-use of the **Muta-ChromoPlate™** method. Smaller sample sizes are required and less reagents are used which is similar to the ISO, however, reversion media mixtures amounts and 96-well microplates facilitate pipetting and sample transfer to incubation step which mirrors the **Muta-ChromoPlate™** Ames test procedure. Tests employ a 48-well assessment format by dividing standard 96 well micro-plates in half, for each sample or control.
This method is a perfect hybrid between facility and efficiency and will be the preferred method depending on sample constituents
An example plate of the Modified ISO method is shown (right).



microbialinsights

Microbial Insights, Inc. (MI) is an environmental biotechnology company specializing in the development and application of cutting edge molecular biological tools (MBTs) to describe and quantify microbial communities.

MI is dedicated to providing superior genetic and chemical diagnostic tools to aid our clients in understanding and managing biological processes for a wide range of areas including environmental remediation, microbial induced corrosion, and microbial source tracking. In 2011 EBPI partnered with Microbial Insights to help market and support Microbial Insights in Canada.

MICROBIAL INSIGHTS 3 MAIN AREAS OF FOCUS ARE:

ENVIRONMENTAL REMEDIATION:

Bioremediation, harnessing the ability of microorganisms to destroy environmental pollutants, is a frequently preferred strategy for treatment of sites impacted by contaminants ranging from petroleum hydrocarbons to chlorinated solvents.
Whether through monitored natural attenuation or engineered approaches, successful bioremediation depends upon the presence, concentration, and activity of contaminant degrading microorganisms.

Bio-Trap (above)
Passive sampling tool that can be used to collect microbial populations for site characterization.

MICROBIAL INDUCED CORROSION:

Microbiologically induced or influenced corrosion (MIC), is a process whereby microorganisms initiate, facilitate, or otherwise accelerate corrosion of metals, affects nearly all industries and can cause severe economic, health and safety, and environmental impacts.
Prevention of microbial induced corrosion requires understanding the dynamics of the entire microbial community and most importantly detection and quantification of the microorganisms responsible so that control measures can be taken.
Microbial Insights offers a variety of molecular microbiological methods (MMMs) for more comprehensive characterization of microbial communities and more accurate quantification of MIC associated microorganisms.

MICROBIAL SOURCE TRACKING:

Fecal contamination of surface waters, which can cause beach closures and outbreaks of waterborne diseases, can stem from a variety of sources including sewage, urban runoff, wildlife, livestock, and agricultural activities.
Microbial source tracking (MST) analyses provide a key advantage over traditional methods - identification of the source of fecal contamination so that control measures can be implemented to reduce fecal pollution and ultimately improve water quality.

MOLECULAR BIOLOGICAL TOOLS

Molecular biological tools (MBT's) and environmental molecular diagnostics (EMDs) encompass a variety of chemical-. biochemical-, DNA- and RNA-based analyses to characterize and quantify microbial populations and activity.

CENSUS – Based on quantitative polymerase chain reaction (qPCR), CENSUS is a nucleic acid-based approach to quantify specific microorganisms, groups of microorganisms, or functional genes involved in bioremediation or other biological processes. CENSUS targets include bacteria and functional genes responsible for biodegradation of chlorinated solvents (i.e. Dehalococcoides analysis) and petroleum products among others.

QuantArray – A hybrid technology combining the highly parallel detection of DNA microarrays with the accurate and precise quantification of qPCR. The QuantArray provides simultaneous quantification of key organisms, critical functional genes and terminal electron accepting processes for more comprehensive yet economical site assessment.

Phospholipid Fatty Acid (PLFA) – A broad-based biochemical approach to assess viable biomass concentrations, profile microbial community composition, and provide insight into the metabolic status or "health" of the microbial community.

Stable Isotope Probing (SIP) – Is biodegradation occurring? SIP is an innovative technique to conclusively demonstrate biodegradation by quantifying incorporation of a 13C labeled contaminant of concern into microbial biomass and dissolved inorganic carbon.

Next Generation Sequencing (NGS) – NGS is a DNA-based technique which generates a profile or "fingerprint" which can be used to identify dominant members of the microbial community.

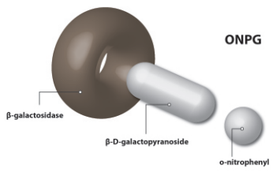
UMU-ChromoTest™

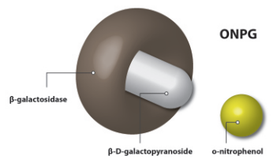
THE PRINCIPLES OF THE UMU-CHROMOTEST

EBPI has developed the UMU-ChromoTest into a simple procedure based upon th International Organization for Standardization protocol ISO 13829 (Water Qualit Determination of the genotoxicity of water and waste water using the **umu-test**), which ca be performed easily in a non-specialized laboratory.

The UMU-Chromo-Test is based on a novel genetically engineered Salmonella *typhimurium* which measures the response of a cell to genetic damage. In just a few hours, the kit provides a clear, quantitative measurement of the genotoxicity of a sample by simple colorimetric evaluation.

The UMU-ChromoTest uses genetically engineered Salmonella *typhimurium* TA1535 (as used in the umu-test) [pSK1002] which are exposed to different concentrations of the samples to be tested. The test is based on the induction of the umuC-gene which is fused to the lacZ-gene which is responsible for the production of β-galactosidase, which can easily be assayed as an indirect measure of DNA damage, or genotoxicity.



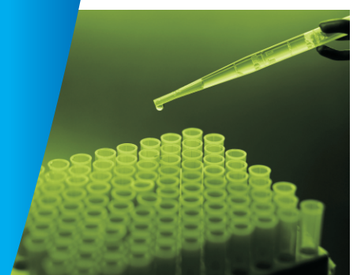


The test uses a single strain of bacteria, however, it can detect a number of different types of mutations, and closely matches results from the traditional Ames test (approximately 90% agreement). The Umu test is rapid, simple, highly reproducible and represents significant reductions in material expenses and labour compared to the Ames test and other methods and has been promoted as a new standard for screening purposes by a number of leading scientists.

MEASURING GENOTOXICITY

The umu-test uses the internationally accepted metric of the Umu Induction Ratio (umu-IR).

The OD_{600} is measured before and after a two hour growth phase in order to identify any possible toxic effects of samples, which may invalidate the results. The readings are used to calculate the Growth Factor which is in-turn used to scale the relative β-galactosidase activity.



The β-galactosidase activity is calculated by comparing the OD_{420} readings of the samples to the negative control.

These two measurements are combined to yield the Induction Ratio, which shows the increase in umu induction of samples relative to the negative control.

TOXI-ChromoTest™

THE PRINCIPLES OF THE TOXI-CHROMOTEST

The Toxi-ChromoTest is a bacterial assay for the detection of toxicity. It is based on the ability of toxic materials and antibiotics to inhibit *de novo* synthesis of the inducible enzyme β-galactosidase in a specially designed strain of *E. coli*. This bacterial strain is highly sensitivity to a wide spectrum of toxic substances such as pesticides, mycotoxins and heavy metals.
The sensitivity of the Toxi-ChromoTest is enhanced by exposing the bacteria to stressing conditions prior to their incorporation into the test kits.

The stressed bacteria are mixed with a cocktail containing essential factors required for the recovery of the bacteria from the stress conditions and a specific inducer for the enzyme β-galactosidase. The ability of the cells to synthesize the enzyme depends on their ability to recover from the stress. The activity of the enzyme is detected by reacting it with a chromogenic substrate, resulting in a visible, easily detectable colour formation which can be measured visually or with a spectrophotometer.
Toxic materials interfere with the recovery of most metabolic functions and thus with the synthesis of the enzyme, resulting in a decreased colour development.

Ames-Express™ Internal Bio-Activation
SOS-Express™ Internal Bioactivation System
UMU-Express™ Internal Bioactivation System

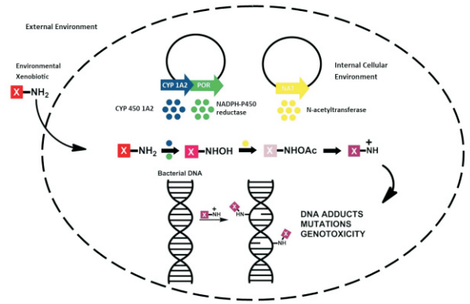
AMES EXPRESS™ BACTERIAL STRAINS

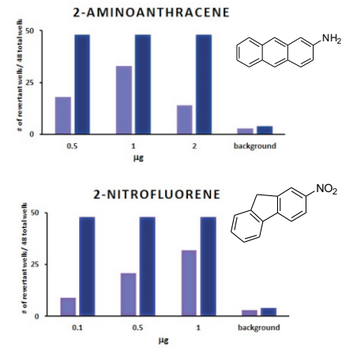
EBPI is proud to officially announce the release of our NEW Ames bacterial strains, the **Ames-Express™** strains.

Ames-Express™ strains are constructed from normal strains (TA100, TA98, TA1535, TA97a, Ta1538), SOS-ChromoTest and the UMU-ChromoTest bacteria, but have been engineered to express either human P450 or GST-theta liver enzymes internally, which promotes bioactivation of xenobiotic molecules into
DNA reactive species in the absence of an S9 mix.
Human recombinant strains that EBPI currently has commercialized are:

Phase 1 Enzymes
CYP P450 1A1 - Polyaromatic Hydrocarbons (PAHs),
CYP P450 1A2 - polycyclic aromatic hydrocarbons (PAHs), Aromatic Amines and Nitroaromatics
CYP P450 3A4 - CYP3A4 is the most common and the most versatile enzymes for drug metabolism
CYP P450 2E1 - Nitrosamines, personal care products

Phase 2 Enzymes
GST T1-1 Haloalkanes, organic thiocyanates,
nitrosoguanides vicinal dihaloalkanes and quinones



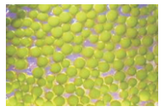


Mutagenic responses for two well known carcinogens.
Tested with traditional Ames bacterial strain **TA98 (purple)**
and new Ames-Express bacterial strains **TA98 P450 1A2 (blue)**
Bacterial strains were analysed using the MOD-ISO procedure.

MicroBioTests Inc.

MicroBioTests Inc located in Gent Belgium, is the developer and manufacturer of the TOXKIT line of products. These kits (like EBPI's line of toxicity testing kits) are used for the detection and quantification of the toxicity of chemicals and or solid and liquid wastes which pollute aquatic and terrestrial environments. All product lines have the unique characteristics that they are culture and maintenance free bioassays, which do not require stock culturing of the test organisms.

EXAMPLES OF THE MICROBIOTESTS LINE OF TOXKITS INCLUDE



Fresh Water and Marine Water Algae Toxicity Test:

Fresh Water ALGALTOXKIT Ftoxicity test is a culture free Selenastrum capricornutum (renamed Raphidocelis subcapitata/Pseudokirchneriella subcapitata), and is a 72 hour toxicity screening test of pure compounds, effluents, sediments, waste waters, and surface and ground waters. It adheres to the protocols for regulatory testing with microalgae, prescribed by international organizations such as the OECD and the ISO.

MARINE ALGALTOXKIT toxicity test contains all the materials necessary to perform two growth inhibition tests with the marine diatom Phaeodactylum tricornutum which is based on the 72 hour assay on growth inhibition of the diatom test species.

The MARINE ALGALTOXKIT toxicity test adheres to the “Marine algal growth inhibition test with Phaeodactylum tricornutum” prescribed by the ISO.

DAPHNIA TOXICITY TESTS:

The **DAPHTOXKIT F** magna contains all the materials necessary to perform six acute toxicity tests with the freshwater crustaceans Daphnia magna. The acute daphnia toxicity tests are strictly adhere to the protocols for regulatory testing with Daphnias prescribed by international organizations such as e.g. the OECD and the ISO.

Like the Daphnia Magna the CERIODAPHTOXKIT F toxicity testing kit contains all the materials necessary to perform six acute toxicity tests with the freshwater crustacean Ceriodaphnia dubia inhibition of the diatom test species.

OTHER TOXKITS MANUFACTURED BY MICROBIOTESTS INCLUDE:

OTHER CRUSTACEANS:

- **RAPIDTOXKIT:** Thamnocephalus platyurus rapid toxicity test (30-60 min) for detection of water contamination
- **THAMNOTOXKIT F:** Thamnocephalus platyurus 24hr toxicity test for screening of pure compound, effluents, sediments, surface and ground waters, biotoxins and cyanotoxins.
- **OSTRACODTOXKIT F:** (Heterocypris incongruens) which is based on a 6 day direct contact for toxicity screening for contaminated river sediments.
- **ARTOXKIT M:** Artemia franciscana toxicity test is a 24h Toxicity Screening assay of Pure Compounds

ROTIFERS:

- **ROTOXKIT M:** Marine/estuarine is a 24h assay based on mortality of the test organisms, with calculation of the 24hLC50.
- **ROTOXKIT F:** Freshwater 24h assay based on mortality of the test organisms, with calculation of the 24hLC50.

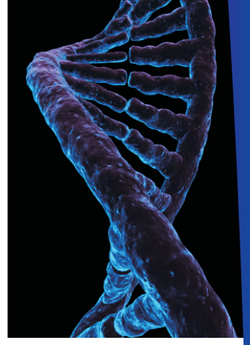
PROTOZOANS:

- **PROTOXKIT F:** Protozoan Ciliate is a 24h growth inhibition toxicity test based on test guideline for Tetrahymena bioassay under priority development in OECD.

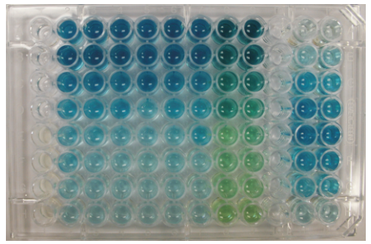
HIGHER PLANTS:

- Phytotoxicity **(PHYTOTOXKIT)** and Germination Toxicity **(PHYTOTESTKIT)** tests
- The Spirodela duckweed Toxkit is a 72 hour growth inhibition of the "first frond" after a 3 day exposure. Growth is determined by measurement of the area of the first frond at T=0 and T=72

SOS-ChromoTest™



The **SOS-ChromoTest** kit is designed for Rapid Detection of Genotoxicity or DNA Damage and utilizes the cell's own mechanisms for the detection of genotoxicity. All living cells have developed a sensitive system for the detection of lesions in their genetic material so that complex enzymatic systems such as the SOS repair system can be activated to repair the damage. Once a lesion has been detected, an SOS promoter is induced to start the transcription of the SOS genes.



- Detects induction of the SOS genes which are involved in DNA repair
- SOS genes are fused in the LacZ reporter gene
- The strain's own repair system was altered by a series of mutations so that even limited damage to the DNA will not be repaired but rather the β-galactosidase enzyme gene expressed.
- Two chromogens are employed for simultaneous detection of β-galactosidase expression and cell viability
- The outer membrane of the cell was modified to increase permeability to many materials.

The SOS-ChromoTest kit is also available in a Chromogenic pad and sediment test form.

NEW

SEDI TOX™

CONTACT SEDIMENT TESTS

The SediTox platform is based on novel bacterial strains also used in EBPI’s line of Toxi-ChromoTest, SOS-ChromoTest and the UMU-ChormoTest products.

The SediTox Kit employs a direct exposure method to assess total responses from all soluble, insoluble, organic, inorganic, and volatile molecules in a given sample without extraction steps.

By employing our genotoxicity strains with this method, EBPI has developed **the first direct contact assay for sub-chronic endpoints like DNA damage.**

BENEFITS OF THE SEDITOX

- Rapid bacterial-based colorimetric bioassay for acute toxicity or genotoxicity .
- The . assay will detect additive, synergistic and antagonistic effects in toxicant mixtures.
- Sensitive to a wide spectrum of toxic (genotoxic) substances.
- The EBPI Express SOS and UMU Strains can be utilized to detect genotoxic potential of metabilites.
- Detection of direct toxicity (genotoxicity) without the need for labour intensive extraction procedures.