CALCULATING THE INDUCTION RATIO:

The umu **Induction Factor** (IR) is a quantitative measure of the gentoxicity of a compound. For each sample dilution, the Growth Factor (to help correct for toxicity) and the β-galactosidase activity (relative units) are calculated and combined to form the IR metric as follows:

Growth Factor (G) = $(A_{600,S} - A_{600,B})/(A_{600,N} - A_{600,B})$

Where A600,S is the absorbance of the sample S at 600 nm
A600, B is the absorbance of the blank at 600 nm
A600, N is the absorbance of the negative control at 600 nm

It is important to note that for an IR to be valid, the growth factor must be above 0.5, that is, the growth has only been inhibited by 50%. If the growth factor is below 0.5, acute toxicity effects may be present and the results maybe skewed invalid for that concentration of sample.

Relative Enzyme Activity (Us) = $(A_{420,S} - A_{420,B}) / (A_{420,N} - A_{420,B})$

Where A420,S is the absorbance of the sample S at 420 nm
A420, B is the absorbance of the blank at 420 nm
A420, N is the absorbance of the negative control at 420 nm

The Induction Ratio is simply the relative enzyme activity, corrected for the amount of toxicity:that concentration of sample.

Induction Ratio (I_R) = (1/G) X U_S

Calculations can be preformed easily with the EBPI analytical spreadsheet found at www.Biotoxicity.com

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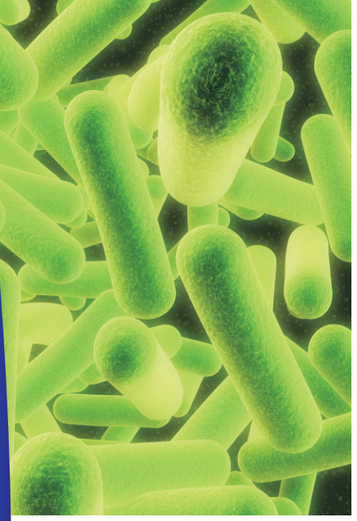
UMU-CHROMOTEST™

Measuring the **Health of the Environment**



UMU-ChromoTest™

THE PRINCIPLES OF THE UMU-CHROMOTEST

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

The UMU-CHROMOTEST 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.

All calculations are easily performed with EBPI's analytical spreadsheets, which are provided for each kit.

The UMU-CHROMOTEST test 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.

VARIATIONS OF THE UMU TEST.

EBPI has developed a few different tests procedures for the umu bacteria.

UMU-test (basic) based on the ISO13829:

The UMU-ChromoTest basic kit is based on the International Standard (ISO 13829) Water Quality - Determination of the genotoxicity of water and waste water using the umu-test.

UMU-test with S9 Activation:

Based on the ISO 13829 test for Water Quality, this test includes all required reagents for the test to be conducted with metabolic activation.

UMU-ChromoTest SP Protocol

A modified version of the UMU-ChromoTest ISO method, the UMU-ChromoTest SP Protocol allows for more test samples to be screened at one time, incorporating less replicates and increasing the number of serial dilutions in a shorter time frame. This allows for up to 11 compounds to be screened in a fraction of the time it takes to perform the full ISO Procedure.

S9 ACTIVATION ENZYMES

As required for reverse mutation bacterial tests, all bacteria should be tested in the presence and absence of an appropriate metabolic activation system.

EBPI utilizes the most commonly used system which is a cofactor supplemented post-mitochondrial fraction prepared from the Sprague-Dawley male rat liver.

The rats are treated with the enzyme-inducing agent Aroclor 1254 prior to the extraction of the S9 fraction from the livers.

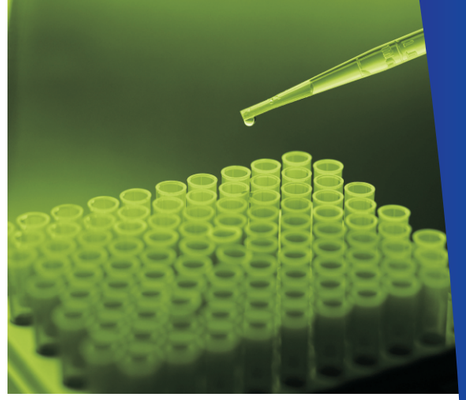
MEASURING GENOTOXICITY

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.



EXAMPLE APPLICATIONS OF THE UMU-CHROMOTEST KIT

- Testing of pharmaceuticals for genotoxic activity.
- Testing of industrial effluents for presence of possible genotoxic compounds.
- Screening of municipal discharges for possible routine presence or spills of genotoxic compounds.
- Screening of surface and ground water for genotoxic residues.
- Screening of potable water supplies for the presence of chemicals with genotoxic potential.
- Screening of water soluble air pollutants for genotoxic agents.
- Evaluation of pure or complex raw mixtures for potential genotoxicity.
- A convenient and easy to use teaching look for university and college laboratories.

CUSTOM SOLUTIONS

At EBPI we strive to meet the demands of our clients and their changing requirements.
For further information:
please contact us at
www.BioToxicity.com

SOS-ChromoTest Kit

Like the umu-test, the SOS-ChromoTest is a microplate genotoxicity bioassay based on the primary response of a genetically engineered bacteria to determine DNA genotoxicity damage using a genetically modified strain of E.*coli*.
Using a battery of bacterial biosensors is an important tool for tool for determination of genotoxicity.