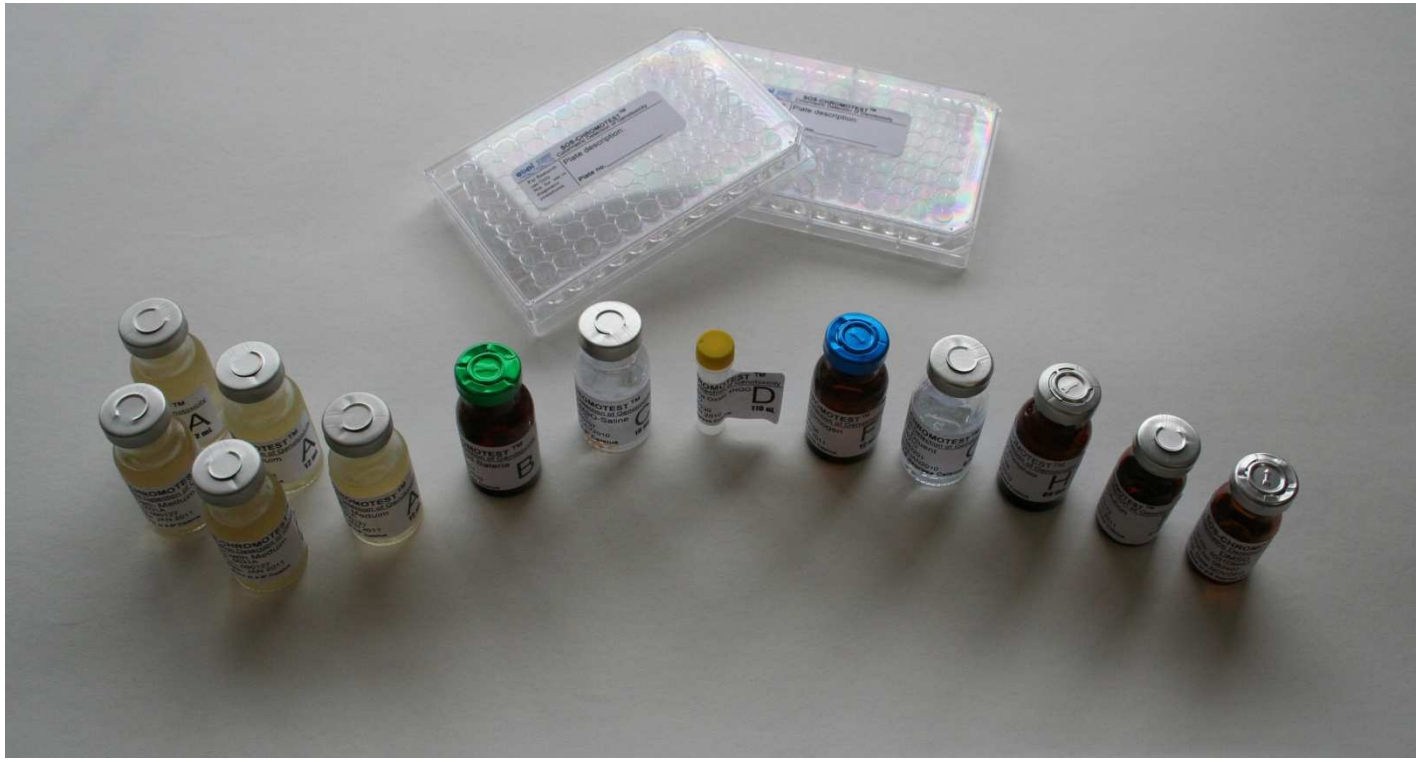
SOS-ChromoTest™

A Users Guide

environmental bio-detection products inc.

SOS-ChromoTest™

Kit Components and Reagents



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SOS-ChromoTest™

- **The SOS ChromoTest™ is a test developed for the detection of genotoxic materials that cause damage to a cell's DNA.**

- Developed to test for the presence of genotoxic materials in sediment, air, chemicals, food components, cosmetics, waste waters, potable waters .

- The test has been designed to both test for the presence of genotoxins in solution and genotoxins that may be transported into cells through direct contact.

- The test Provides a colorimetric endpoint through which the presence of genotoxic materials can be determined and when done in solution and absorbance measured, allows the calculation of an SOS induction factor (SOSIP) or slope factor by which the relative strength of genotoxic compounds or mixtures can be determined.

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- The test employs a mutant PQ37 strain of E. coli in which the SOS gene complex repair promoter region of the genome that is responsible for activating the SOS genes has been linked to the β gal gene responsible for the production of the β-galactosidase enzyme.

- The degree to which the cell is trying to repair DNA damage using the SOS gene repair complex is now directly linked to the production of β-galactosidase which is measured by the enzyme's reaction with a blue chromogen.

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SOS-ChromoTest™ Kit Components

- Growth Media for the SOS-ChromoTest™ Bacteria (5 units)
- The SOS-ChromoTest™ bacteria (1 unit)
- Saline Solution (1 unit)
- Standard Genotoxic Solution – 4NQO (1 unit)
- Blue Chromogen Solution (2 units)
- Diluent for Alkaline phosphatase Substrate (1 unit)
- Dried Alkaline Phosphatase Substrate (1 unit)
- Stop Solution (1 unit)
- DMOS solution for dissolving water insoluble materials (1 unit)
- 96 Well Micro-Plates for assays (2 units)

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SOS-ChromoTest™ S9 Activation Enzymes

S-9 Activation Mix Components (optional)

- S9A $MgCl_2$ + KCL
- S9B Glucose-6-Phosphate
- S9C NADP
- S9D Tris HCL Buffer
- S9E Sterile Distilled Water
- S9F Rat Liver Extract
- 2AA Positive Controls

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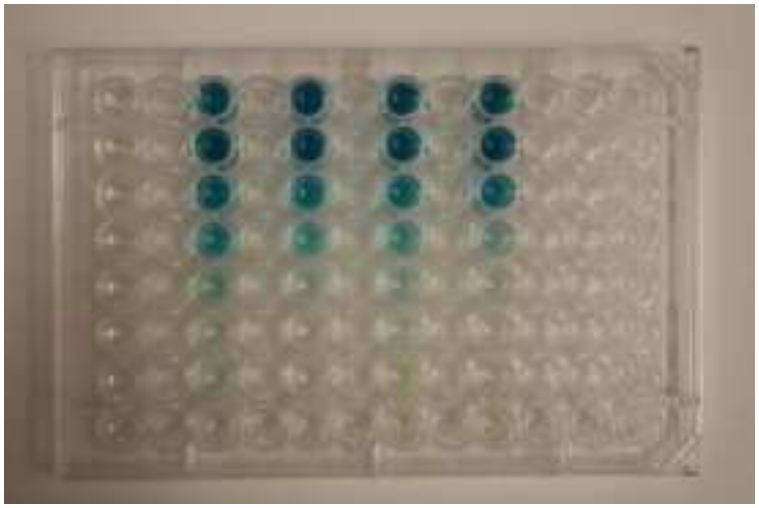
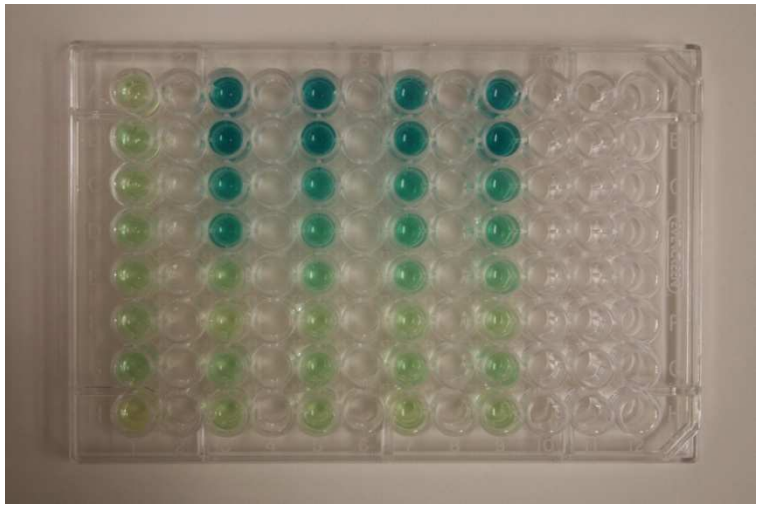
- The SOS-ChromoTest™ provides a clear colour endpoint.
- Reagents, cultures and other consumable components are supplied ready-to-use in a non-specialized laboratory.





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- No need for Cultures

- Quick and Easy Overnight Growth of the Bacteria.

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- Although Sterilization of the Samples is generally not required, because of the short time needed for the test it may be appropriate under circumstances where significant bacterial contamination is present.

Filter Sterilization of the sample for assays

Filter sterilization of the samples is recommended but not required to be preformed prior to starting the assays. This can be done with either a 0.22µm filter unit, or with a 0.22µm syringe filter (Included upon request).

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Overnight Growth of the Bacteria

1. Remove the vial of Growth Media from the fridge and remove the vial of bacteria from the freezer.

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2. Using aseptic techniques open vial A (Growth Media) and vial B (Bacteria). Transfer the contents from vial A into vial B.

Place the lyophilized stopper back on the vial that now contains the bacteria and growth media and give the bacteria a quick shake to ensure they are well mixed. Incubate overnight At 37^{o}C for 16 to 18 hours

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4. Visually examine the vial containing the bacteria grown overnight for turbidity.

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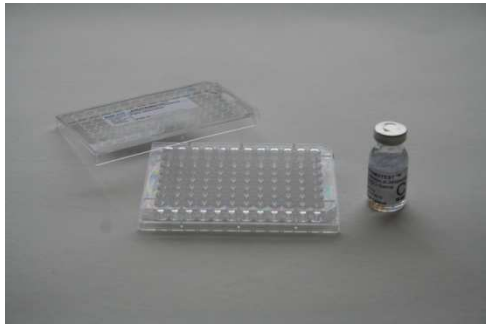

1. Obtain one 96 well micro-plate and vial C (Saline Solution)

2. Dispense 10µl of the Saline Solution to all wells that will be used during the assays.

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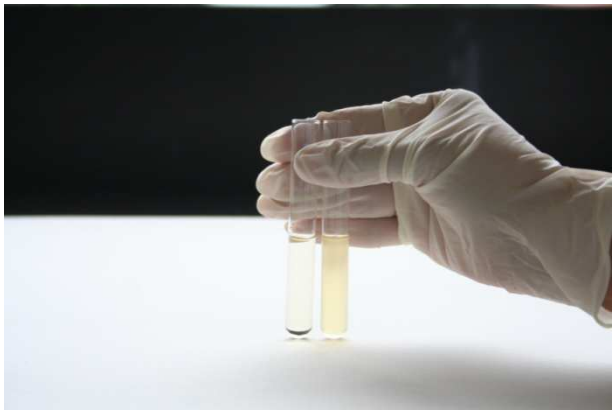
1. Obtain the 4NQO (Control) from the freezer, as well as all samples for assays. Dispense 20µl of the 4NQO solution into well 2A.

2. Remove 10ul from well 2A and dispense into well 2B, mix well and remove 10µl in dispense into well 2C. Continue performing a double dilution until well 2G, remove 10µl from well 2G and discard (Well 2H is a reference well and does not contain any samples or controls).

3. Obtain the sample or samples to be tested, and dispense and double dilute in the same manner as the control (again, row H is a reference row and Does not contain any sample materials).

Preliminary Dilution of the Bacteria and Density Check.

- Take the bacterial suspension and measure the OD (at 600 nm) of the suspension against a fresh medium blank in a 1 cm light path cuvette. Calculate the volume of suspension required to obtain 10mL of bacterial suspension with a final OD600 of 0.05 as outlined in the protocol.



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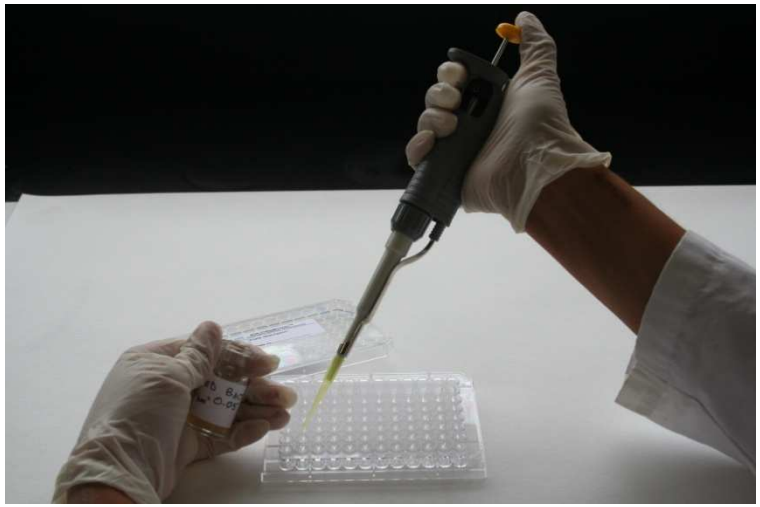
- Once the correct OD(600) of the bacteria has been completed, dispense 100µl of the diluted bacteria to each well of the micro-plate that will be used in the assay (Including row H)



- Incubate the micro-plate at 37^{o}C for 2 hours. During this time, the bacteria are exposed to the material which contain the suspected genotoxins.

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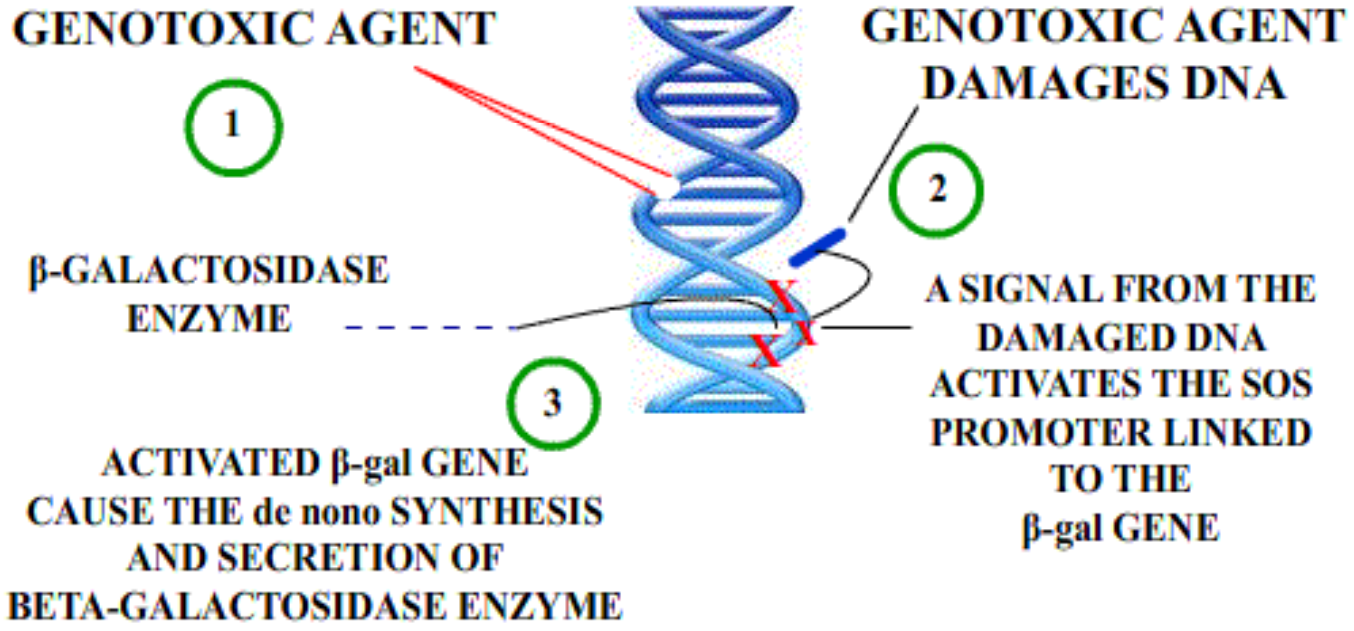
CELLULAR EVENTS IN SOS BACTERIA

WHEN EXPOSED TO A GENOTOXIC AGENT



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- After the two hour incubation in which the bacteria have been exposed to the samples; a Chromogenic substrate will be added.

- There are two procedures.

    - A) Simultaneous Activity Check of β-galactosidase and Alkaline Phosphatase (analysis by instrumentation)

    - B) Sequential Activity Check of β-galactosidase and Alkaline Phosphatase (visual analysis)

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Simultaneous Activity Check of β-galactosidase and Alkaline Phosphatase

1. Transfer the Blue Chromogen from bottle F to the dry Alkaline Phosphatase substrate in bottle H and mix well.
2. Add 100µl from bottle H into each well of the plate.
3. Incubate the plate at 37^{o}C for 60 to 90 minutes until a green colour appears.
4. Add 50µl of the stop solution in bottle I to each well of the plates.

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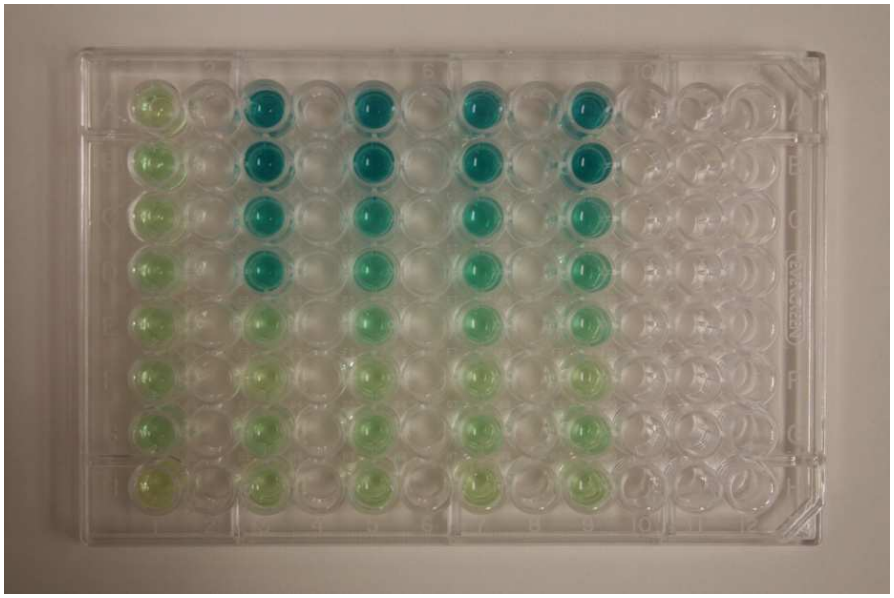
- Read absorbance (optical density) at 615nm to measure genotoxic activity.
- Read absorbance at 405nm to determine viability of bacteria.



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Sequential Activity Check of β-galactosidase and Alkaline Phosphatase (visual analysis)

1. Add 100µl of Blue Chromogen from bottle F into each well of the plate.
2. Incubate the plate at 37°C for 60 to 90 minutes until blue colour develops. If no positive colour reaction is noted, perform a viability check.

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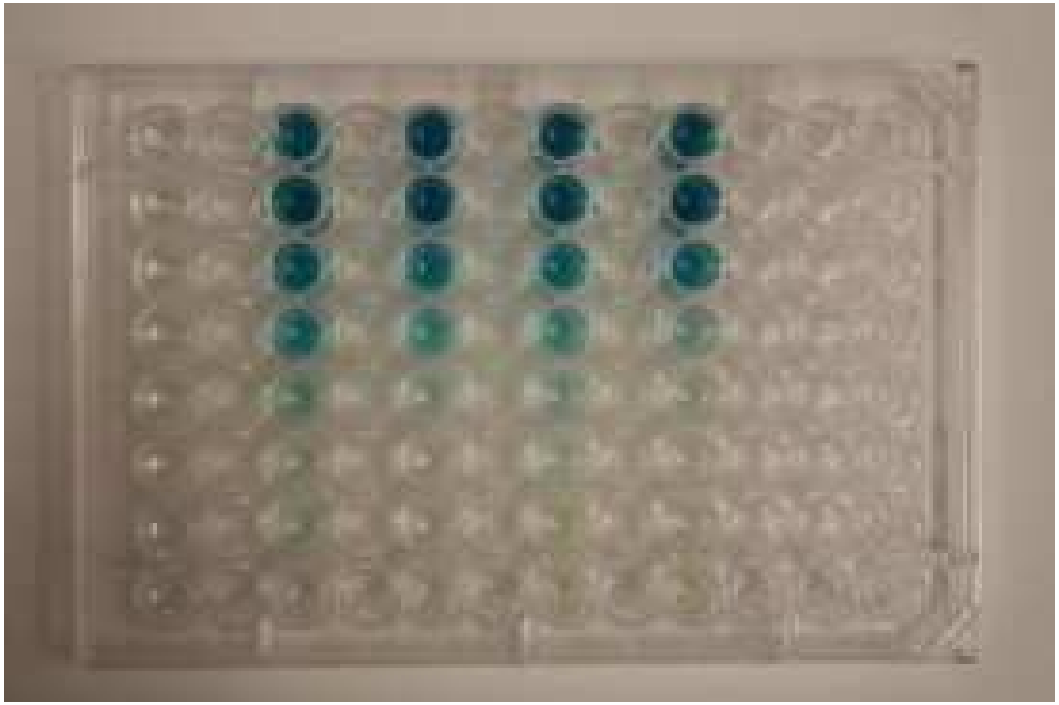
Sequential Activity Check of β-galactosidase and Alkaline Phosphatase (visual analysis)



Read absorbance (optical density) at 615nm to measure genotoxic activity

Viability Check – If no colour development

a) Transfer the diluent from bottle G into bottle H containing the dry Alkaline Phosphatase substrate and mix well.
b) Add 50µl from bottle H into the wells of the test sample dilutions.
c) Incubate the plate at 37^{o}C for 30 to 60 minutes, until a yellow colour develops in wells containing bacteria without genotoxic materials (test blank).
d) Add 50µl of Stop Solution (bottle I) to wells if desired.
e) Examine the wells for development of yellow colour signifying viability of bacteria.

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Calculating the SOSIP

Identify the positively linear portion of the plot, i.e., the OD (measured at 615 nm) increases linearly with the concentration of tested material (the line between the concentrations (OD1) and (OD3) ug/ml in Figure 1). The SOS Inducing Potency (SOSIP) is simply the slope of the linear portion of the plot and is given in the following equation:

(1) SOSIP = 10 X (OD1 – OD3)/(C1 – C3)

The expression "(C1 – C3)" in equation (1) is entered in nano-moles per reaction well. Equation (2) transforms microgram concentration values to the required nano-mole unitage:

(2) C = CONC X VOL / MW

where:

CONC -concentration of tested material in µg/ml,
VOL -volume of the tested material solution in the well expressed in micro-litres
MW -molecular weight of the tested material.

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- Since the calculated SOSIP may change from time to time due to changing incubation conditions, age of the bacteria etc, it would be wise to correct the values according to the activity of a known standard. The suggested procedure is as follows:

- Divide the obtained SOSIP by 71 to get a "SOSIP correction factor“(71 is the published value for the 4NQO in the original procedure). Divide all SOSIP values obtained for the tested materials by the "correction factor", to arrive at a value comparable to previously published values.

- Divide all SOSIP values obtained for the tested materials by the "correction factor", to arrive at a value comparable to previously published values

SOS-ChromoTest™

- The SOS-ChromoTest Kit™ is available with or without the S9 Activation Enzymes.
- The S9 Activation Enzymes are from the male Sprague-Dawley Rat liver –Aroclor 1254 inducted.

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