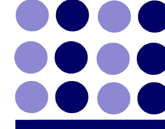
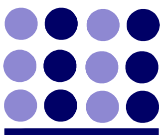


Users Guide



Kit Contents:

Standard Reagent List (TA100)

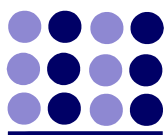
- A: SOS Growth medium
- B: SOS Bacteria strain
- C: 10% DMSO in saline, diluent
- D: Standard genotoxic solution (4NQO)
- F: Blue chromogen solution
- G: Diluent for alkaline phosphatase substrate
- H: Dried alkaline phosphatase substrate
- I: Stop Solution
- DMSO

Disposable list

- **96-well plate (2 units)**
- **Reagent boats (10 units)**
- **A membrane filter (0.22 µm)**
- **A disposable biohazard bag**

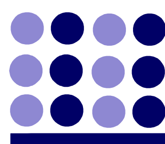
Required Equipment

- **Micropipette using disposable sterile tips in the range of 5 µl to 1000 µl**
- **An eight-channel multi-pipette (50 to 200 µl)**
- **Vortexer (optional)**
- **Microplate Reader**
- **A 37˚C incubator**



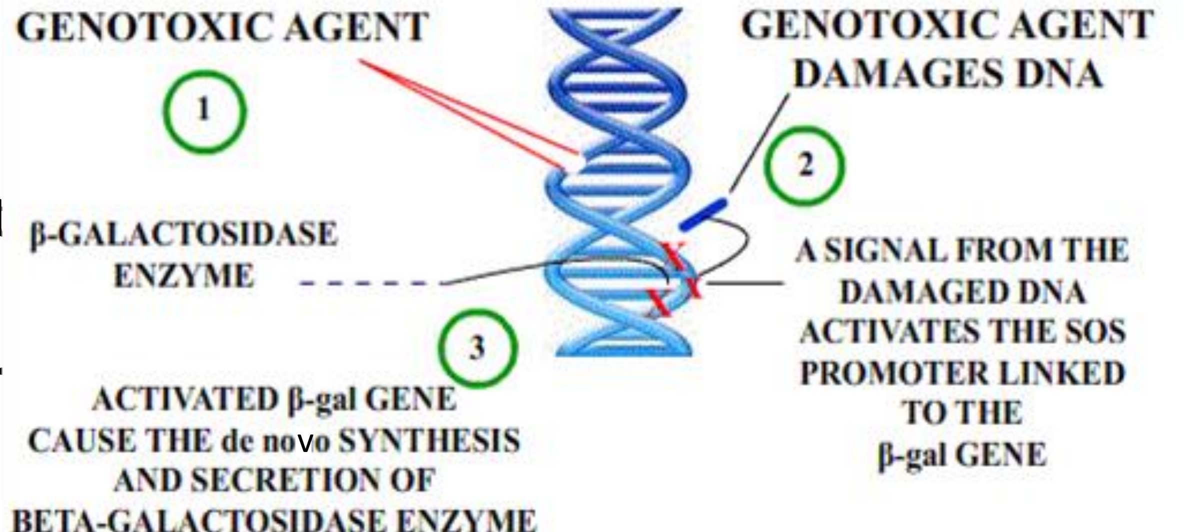
Applications:

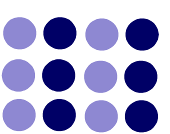
- Testing of industrial effluents for presence of possible genotoxic compounds.
- Screening of municipal discharges for spill contamination, improper chemical disposal.
- Routine monitoring of waste water effluent for quality and genotoxicity.
- Screening of recycled potable water supplies for presence of priority pollutants and genotoxins.
- Evaluating water samples for elevated levels of personal care product (PCP) residues.
- Effective teaching tools for University and College laboratories to demonstrate concepts like metabolism, carcinogenesis and mutation.
- Understanding the effects of genomic integrity.



Engineering of the Strain

- Detects induction of the SOS genes which are involved with 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 B-galactosidase enzyme gene is expressed.
- The outer membrane of the cell was modified to increase permeability to many materials.





1. Rehydrate bacteria and incubate at 37°C overnight.

A → B

Mix →

B A (1 ml)

Incubate overnight at 37°C →

2. Measure absorption of 600 nm ± 20 nm light. Dilute bacterial suspension to give optical density = 0.05.

A + A (Fresh vial) → 0.05

OD = 0.05

Reagent blank
Positive control
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5

C D 1 2 3 4 5

3. Prepare the plate by performing serial dilutions for the positive control and all samples.

Negative control (row H)

←

0.05

4. Add 100 µL of diluted bacterial suspension to every well except for those containing reagent blank.

Incubate at 37°C for 2 hours.

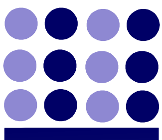
F

5. Add 100 µL of chromogen to each well.

Incubate at 37°C for 30 minutes. →

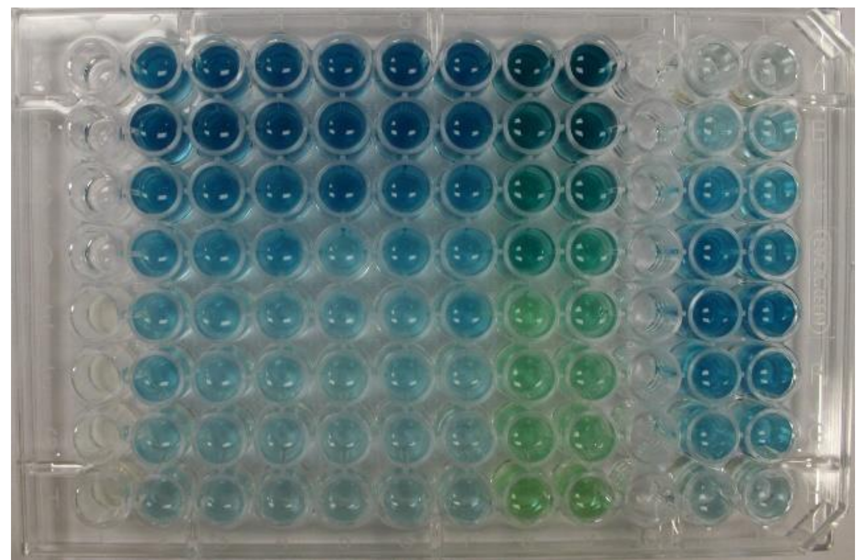
→

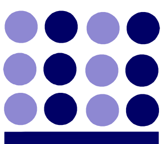
6. Evaluate results visually and/or using a plate reader. Perform results analysis using EBPI's bioinformatics spreadsheet.



SOS-ChromoTest™ Advantages

- Reagents, bacteria and other consumable components are supplied ready-to-use in a non-specialized laboratory
- Assay endpoint is a highly sensitive colorimetric change that is easily interpreted
- Small sample volumes are employed (10 µL).
- SOS-ChromoTest™ is rapid, test is done in one day.
- 20 samples can be run with each kit with 8 dilutions.



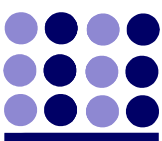


Bioactivation (S9 kit add-on)

- Many genotoxins must first be metabolized into their reactive form by enzymes
- Depending on the compound under study, bioactivation may be required for detection
- EBPI offers traditional methods of pro genotoxin activation through the addition of S9 liver fraction
- EBPI employs a commonly used metabolic activation system which includes post-mitochondrial liver fractions isolated from Sprague Dawley rats, supplemented with cofactors
- The rats are pre-treated with Aroclor 1254 to stimulate enzyme production prior to liver extraction
- This option is offered with all mutagenicity and genotoxicity testing kits for a supplemental cost

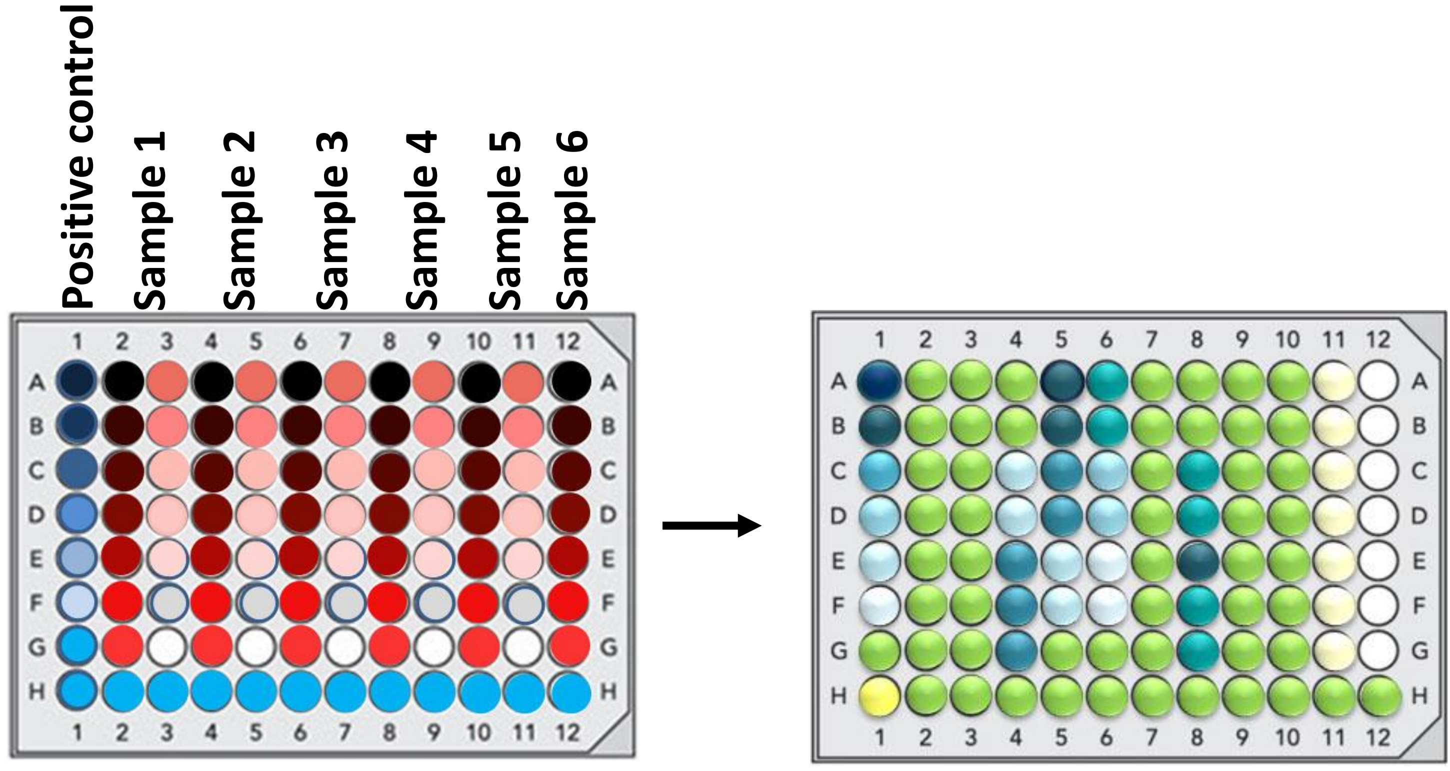
S9 Kit Contents

S9A: KCL: MGCl2
S9B: Glucose-6-phosphate solution
S9C: NADP solution
S9D: Phosphate buffer
S9F: Lyophilized S9 fraction
2AA 2-Amino-Athracene

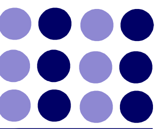


SOS-ChromoTest™ Procedure

Experiment overview



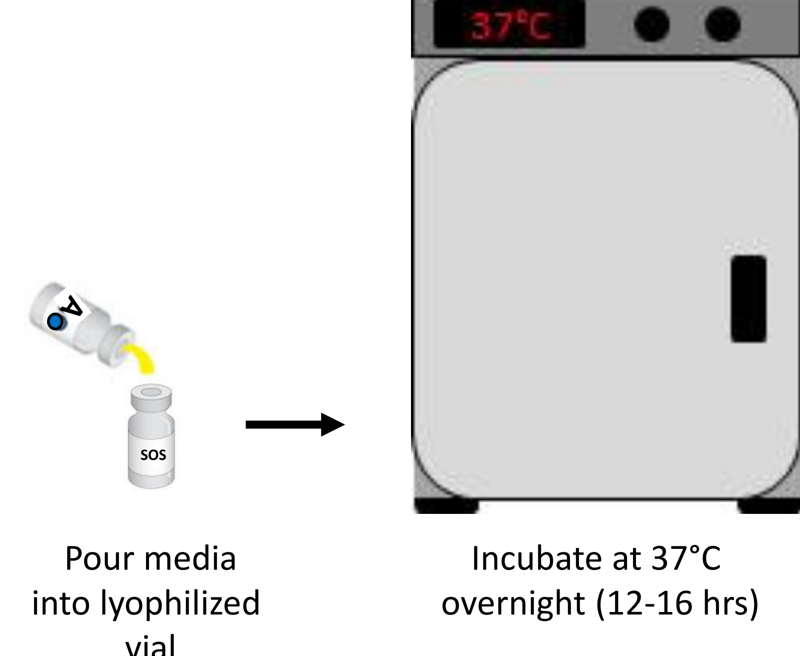
Note: Prior to using our test kits, we highly recommend the development of individual outlines that are representative of the respective experiment. This outline is only provided as a guideline for one possible method



Detailed Procedure

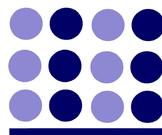
1. Overnight inoculation and initial bacterial growth

- Always use aseptic techniques for all steps in this procedure
- Add SOS growth media to bacterial bottle
- Shake to dissolve and place in incubator overnight at 37 °C with shaking (if possible)
  - aeration will aid bacterial growth.



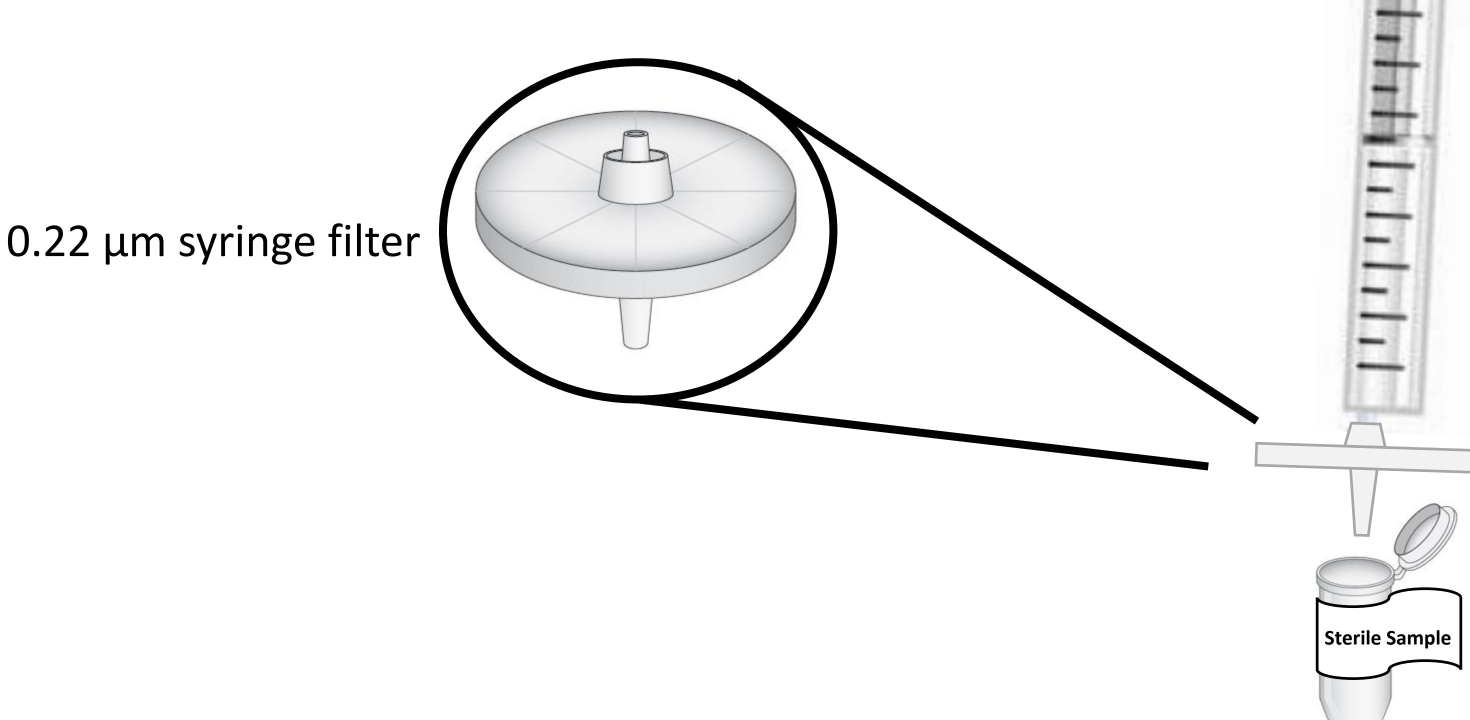
Pour media into lyophilized vial

Incubate at 37°C overnight (12-16 hrs)

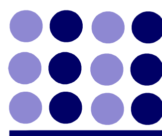


2. Sample preparation

- Filter sterilize the sample to be tested using a 0.22 µm membrane filter.
- Prepared sample dilutions, in sterile distilled water
- Volume of each tube should be 1 ml

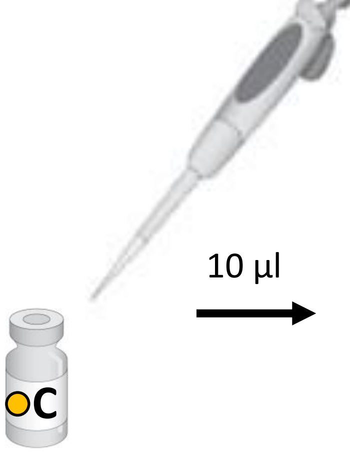


Filter sterilize samples using filter unit (large volume) or syringe filter (small volume).

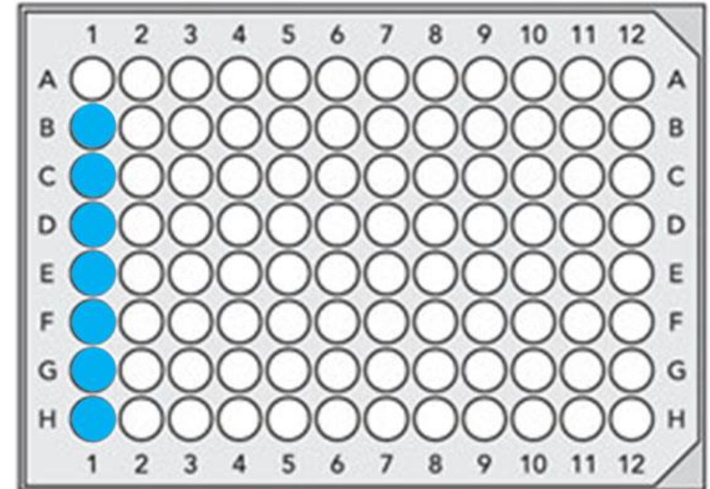


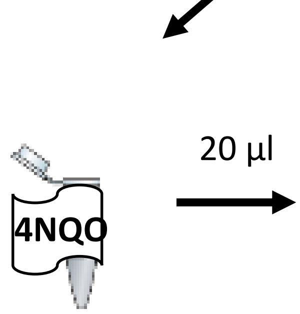
3. Preparation of positive control column

- Add 10 µl of DMSO diluent B1-H1
- Add 20 µl of 4NQO to A1 and follow up with a serial dilution with column 1.

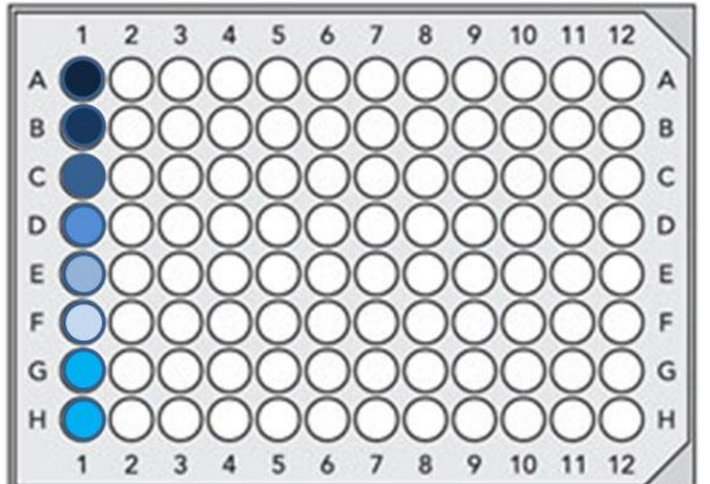


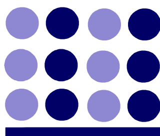
10 µl





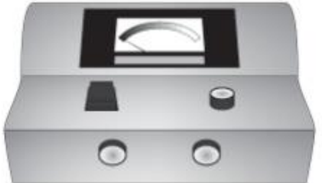
20 µl



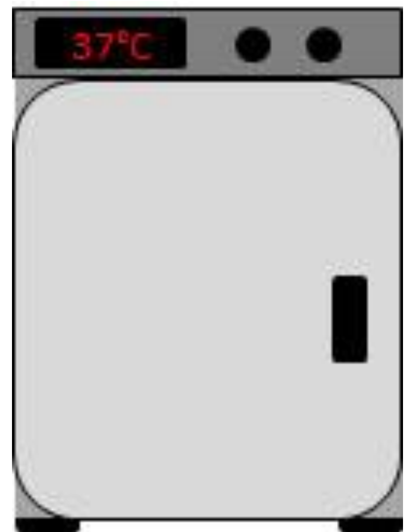


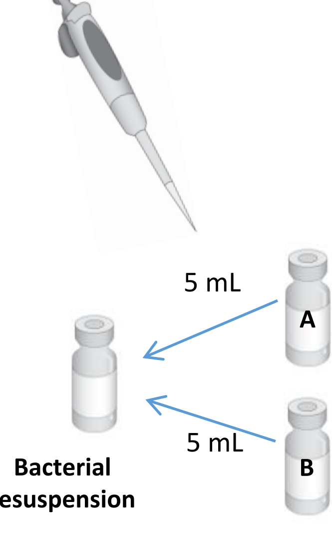
4. Bacteria dilution and Resuspension

- The next morning observe bacterial vial for turbidity
- If turbidity is seen proceed with initial OD_{600} measurement
- If turbidity is not seen continue growth in incubator
- OD_{600} should be around 0.1 after overnight growth
- Calculate dilution volume to prepare bacterial resuspension with a final OD_{600} of 0.05
    - Bacterial dilution encourages log growth phase and increases uptake of solution components

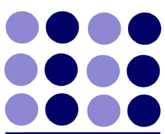


2. Measure absorption of 600 nm ± 20 nm light. The absorption (OD) should be ≈ 0.1



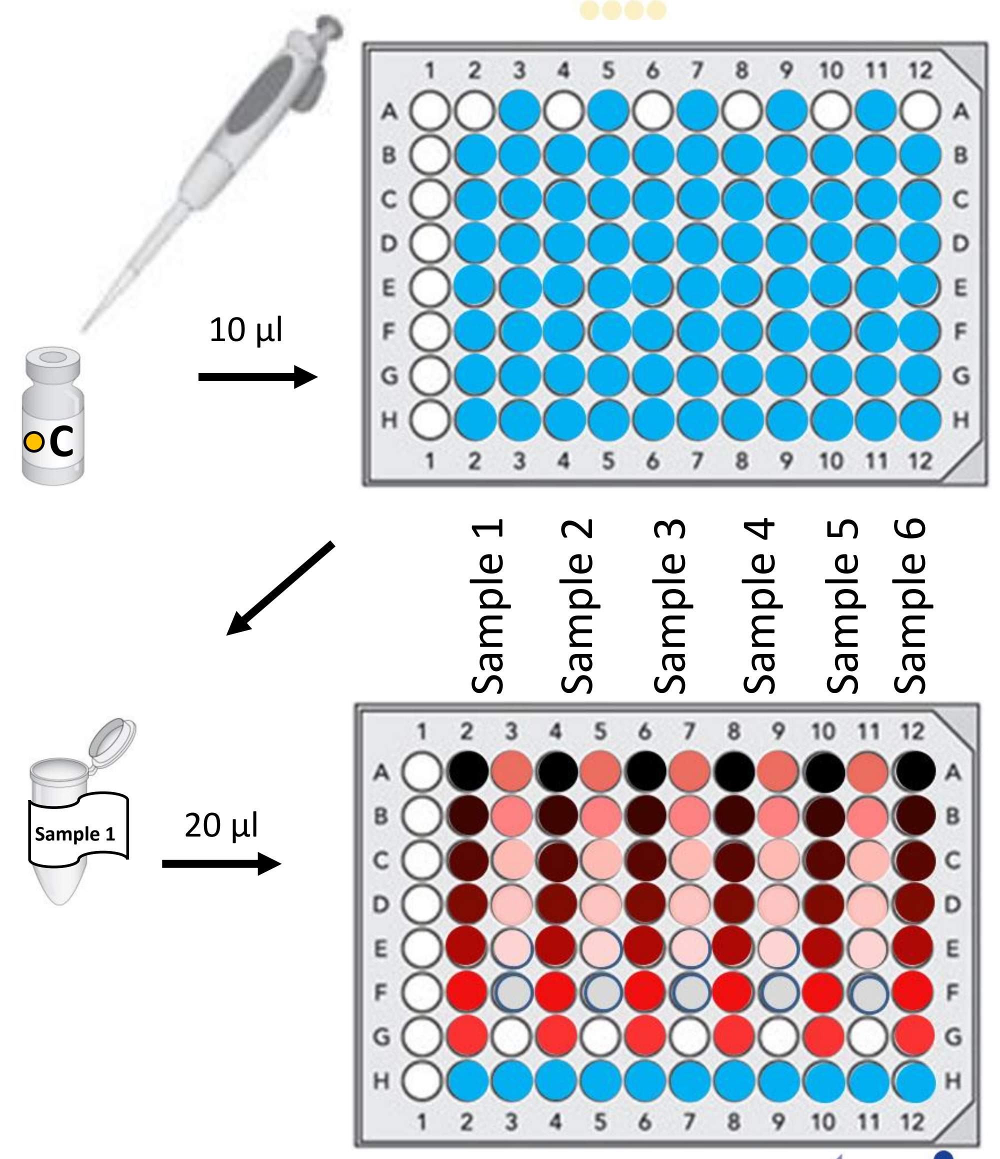


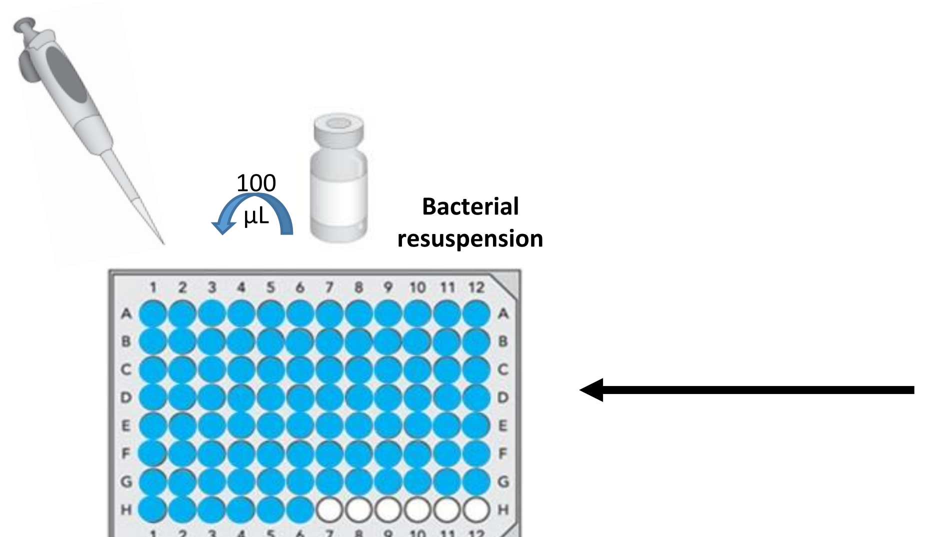
Calculate dilution volume to obtain a final OD_{600} of 0.05

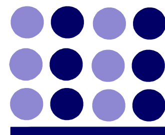


5. Add samples to plate

- Add 10 µl of DMSO diluent in plate **(except for A2, A4, A6, A8, A10, A12, Column 1)**
- Add 20 µl of samples respectively into wells **(A2, A4, A6, A8,A10, A12)**
- Perform serial dilutions of the samples down the respective columns
- Add 100µl of re-suspended bacteria to each well excluding a machine blank (if included)

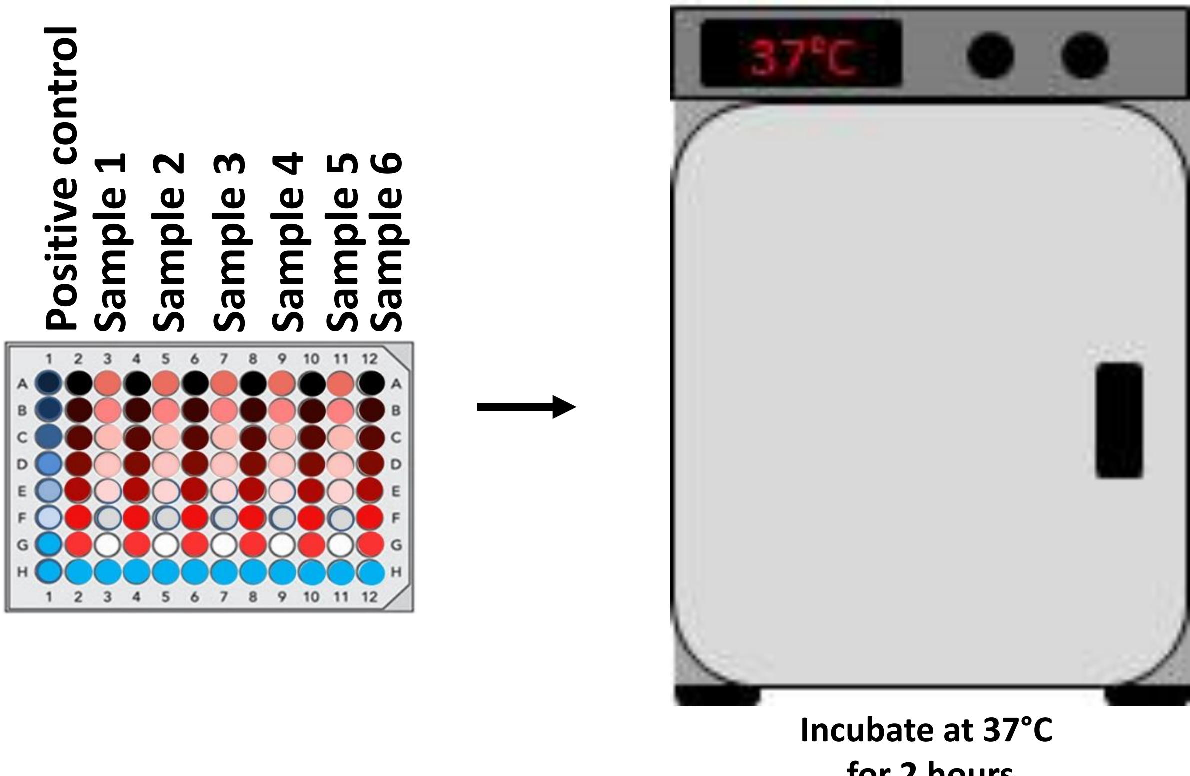


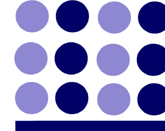




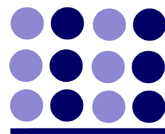
6. SOS incubation

- Incubate plate at 37°C for 120 mins





Simultaneous Activity Check of β-galactosidase and Alkaline Phosphatase Step



7. Add Blue Chromogen and Alkaline Phosphatase to plate

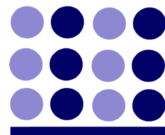
- Add Blue Chromogen into alkaline phosphatase bottle and mix
- Transfer 100 µl of the mix to each well
- Incubate for 60-90 minutes at 37˚C

H

Positive control
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6

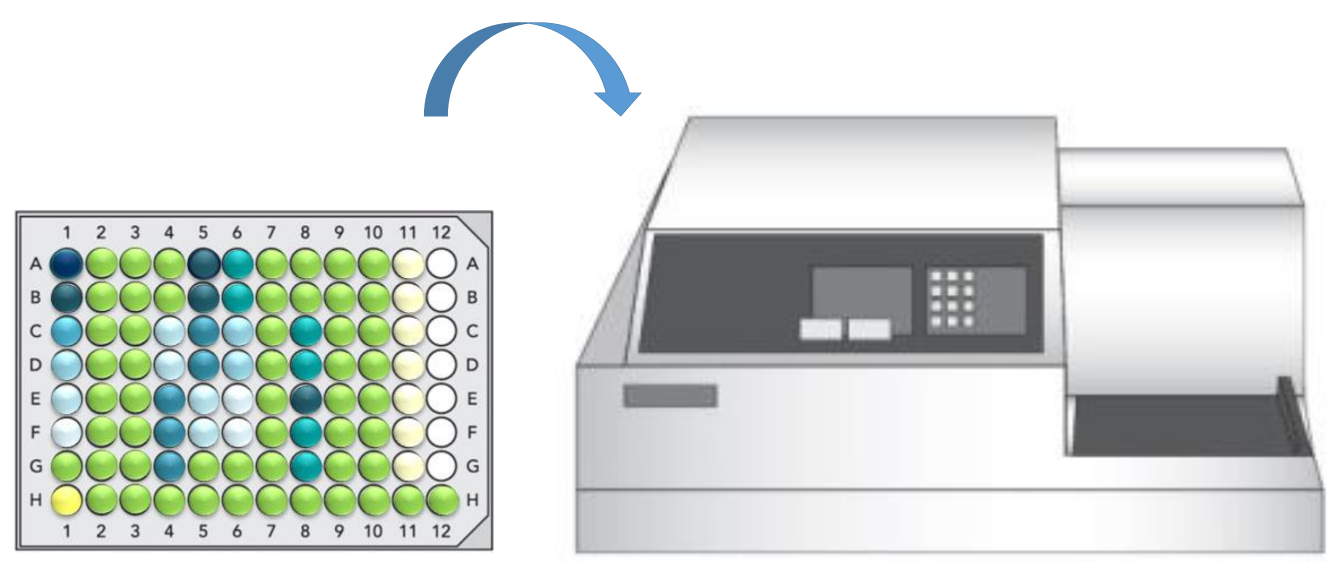
37°C

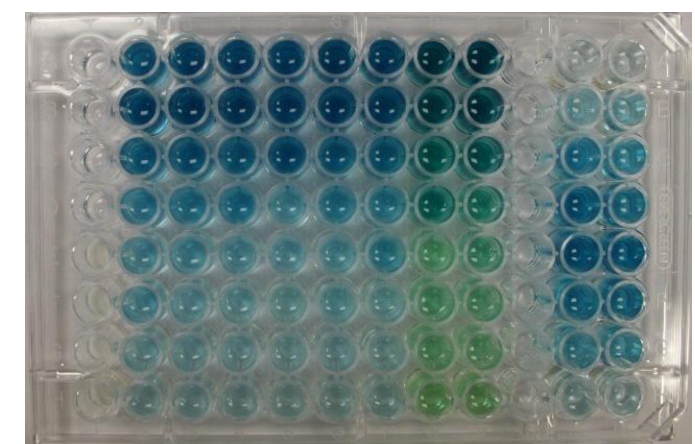
Incubate at 37°C
for 60 - 90 mins

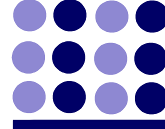


8. Plate reading

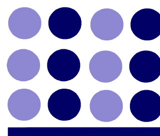
- Read absorbance at 420 nm (±20) nm to determine viability
- Read absorbance at 615 nm (±20) nm to determine genotoxicity





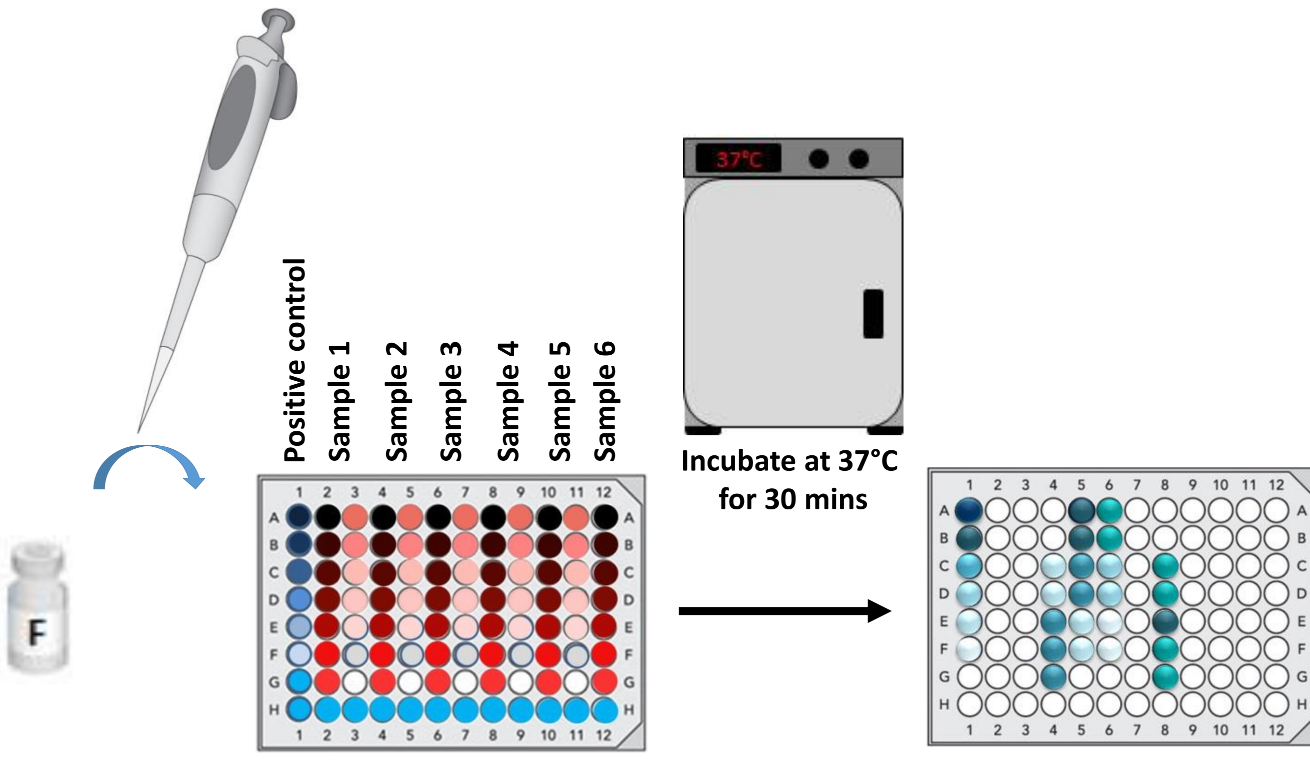


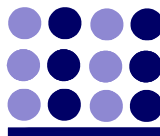
Alternative: Sequential Activity Check of β-galactosidase and Alkaline Phosphatase



7. Add Chromogen to plate

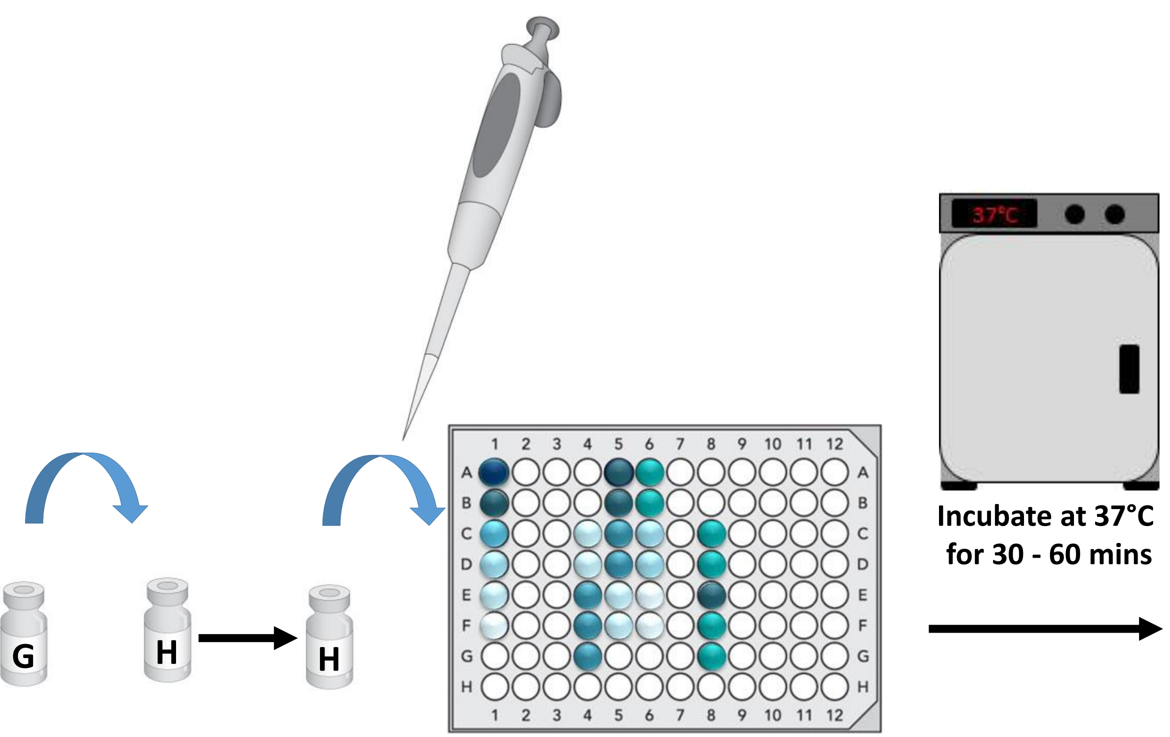
- Transferring 100 µL of Bottle F (Blue Chromogen) to plate
- Incubate for 30 mins at 37˚C

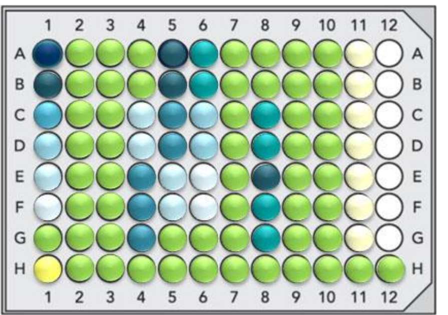


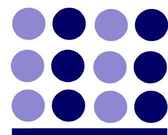


8. Add mixture to plate

- Mix diluent bottle G with alkaline phosphatase bottle H
- Transferring 50 µL of mixture to plate
- Incubate for 30-60 mins at 37˚C

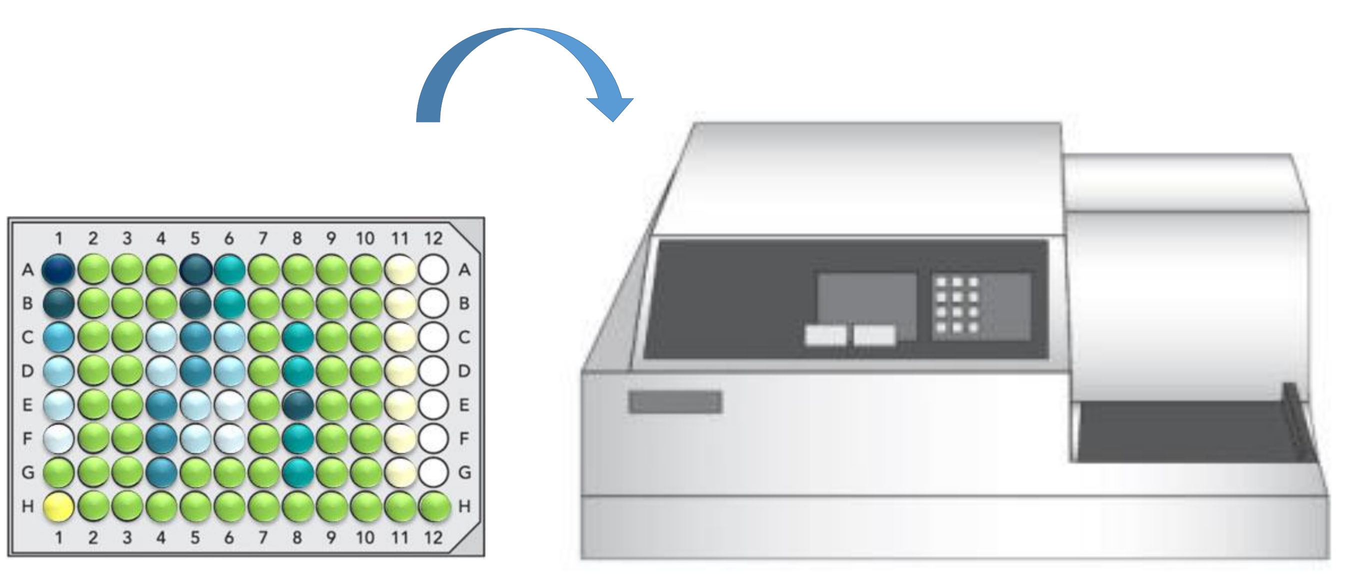


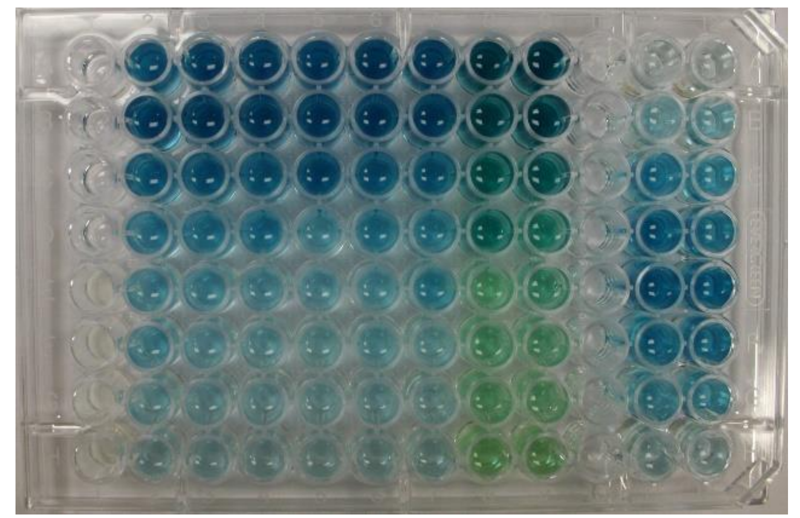


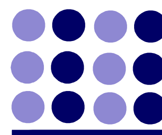


9. Plate reading

- Read absorbance at 420 nm (±20) nm to determine viability
- Read absorbance at 615 nm (±20) nm to determine genotoxic activity







Calculating the SOSIP

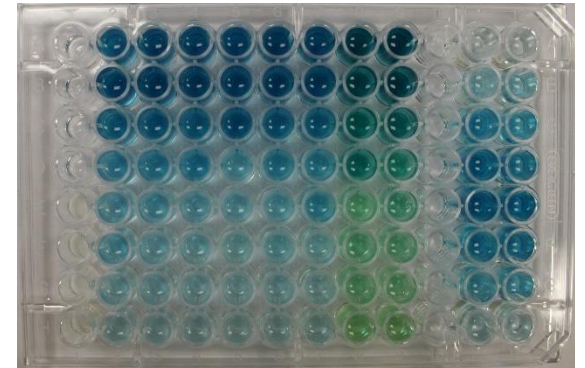
Identify the positively linear portion of the plot, i.e., the OD (measured at 615 nm) increases linearly with the concentration of tested material (the line between the concentrations (OD1) and (OD3) ug/ml in Figure 1). The SOS Inducing Potency (SOSIP) is simply the slope of the linear portion of the 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 nanomoles per reaction well. Equation (2) transforms microgram concentration values to the required nanomole units:

(2) C = CONC X VOL / MW

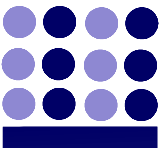
where:



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.

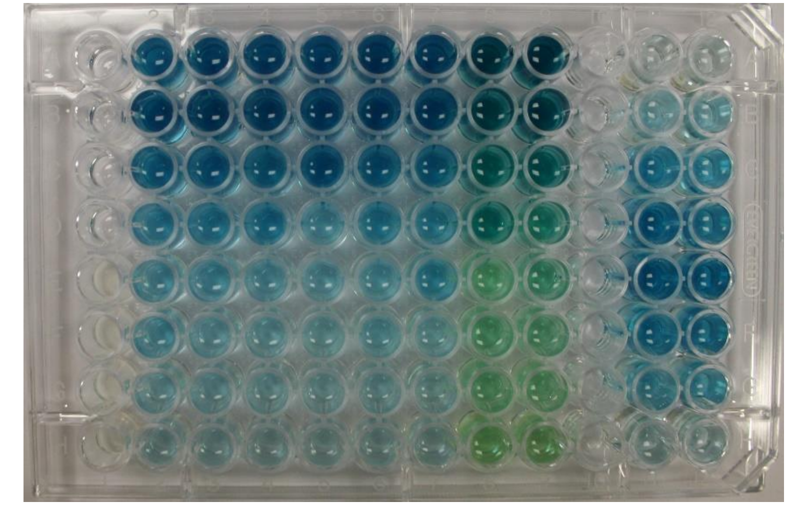


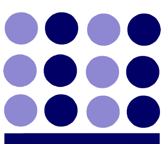
Controlling Variations in Growth

- 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.

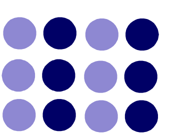
- Divide all SOSIP values obtained for the tested materials by the "correction factor", to arrive at a value comparable to previously published values





SOS-Chromotest EXCEL Spreadsheet

ebpi environmental bio-detection products inc.



1. Rehydrate bacteria and incubate at 37°C overnight.

B → Mix → B A (1 ml)

Incubate overnight at 37°C

2. Measure absorption of 600 nm ± 20 nm light. Dilute bacterial suspension to give optical density = 0.05.

A + A (Fresh vial) → 0.05

OD = 0.05

Reagent blank | Positive control | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5

C D 1 2 3 4 5

3. Prepare the plate by performing serial dilutions for the positive control and all samples.

Negative control (row H)

0.05

4. Add 100 µL of diluted bacterial suspension to every well except for those containing reagent blank.

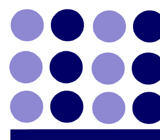
Incubate at 37°C for 2 hours.

F

5. Add 100 µL of chromogen to each well.

Incubate at 37°C for 30 minutes.

6. Evaluate results visually and/or using a plate reader. Perform results analysis using EBPI's bioinformatics spreadsheet.



Kit Options

- Basic Kits with and without or without S9 Activation
- Reagents Only Kits
- All kits/reagents can be modified to meet your requirements
- SOS Express Strains