UMU-ChromoTest™

Users Guide

environmental bio-detection products inc.

Kit Contents:

Reagent List

- A: Growth medium for the UMU-ChromoTest™ bacterial strain 10 mL (5 unit
- B: UMU-ChromoTest™ freeze-dried bacteria 0.2 g (1 unit)
- C: 1x Glucose 1.5 mL (1 unit)
- D: 4-NQO – positive control 100 µL (1 unit)
- E: 10x Concentrated Growth Media 2.5 mL (1 unit)
- F: 10x Concentrated Glucose 100 µL (1 unit) (May require rehydration)
- G: Phosphate Buffer 5 mL (1 unit)
- H: ONPG (1 unit)
- I: B-Buffer 13 mL (1 unit)
- J: 2-Mercaptoethanol 100 µL (1 unit)
- K: Sterile Distilled Water 12 mL (1 unit)
- L: Stop Solution 12 mL (1 unit)

Required Equipment

- Micropipettes with sterile tips in the range of 10 to 200 µL
- Incubator capable of holding a steady temperature of 37 °C
- Spectrophotometer or a photometer equipped with 600 nm filter using 1 cm light-path rectangular cuvettes (for preparation of the bacterial suspension).
- Microplate reader (ELISA Reader) equipped with 600 (± 20) nm and 420 (± 20) nm filters.

Disposable list

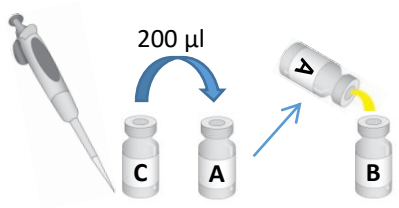
- 2- 96 well microplates
- Kit instructions
- Biohazard bag for disposal of used components.

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.
- Screening of recycled potable water supplies for presence of priority pollutants and genotoxins.
- Screening air particulate mater (PM) for sub chronic human health effects.
- Evaluating water and soil samples for elevated levels of personal care product (PCP) residues.
- Screening of new drug development.

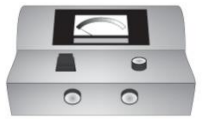
UMU-ChromoTest™

1. Rehydrate bacteria.



Mix and incubate at 37°C overnight

2. Measure absorption of 600 nm ± 20 nm light. The absorption (OD) should be ≈ 0.5.

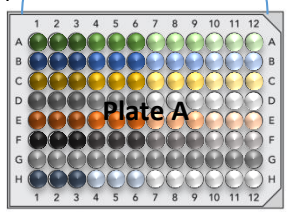


3. Re-inoculate 5 mL of bacterial suspension in new growth medium.

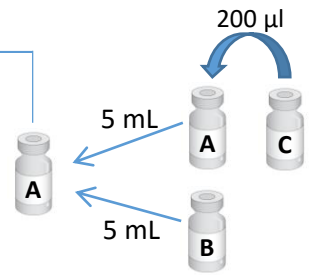
Incubate Plate A at 37°C for 2 hours

4. Prepare Plate A during bacterial suspension incubation by performing serial dilutions for all samples. Plate A will also include all controls (positive, negative, solvent) and blanks. After incubation, dispense 70 µL of bacterial suspension to all appropriate wells.

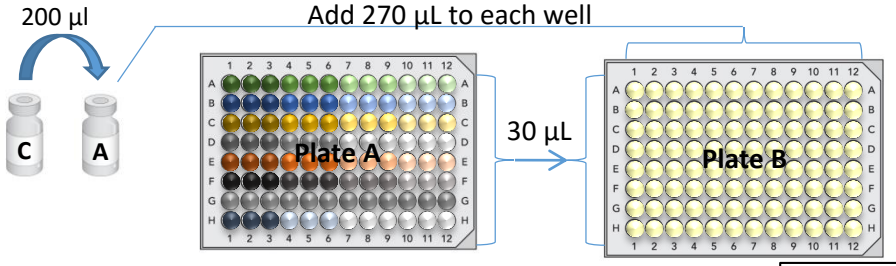
Add 70 µL to all appropriate wells



Incubate at 37°C for 1.5 hours



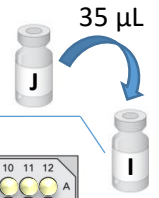
5. Prepare Plate B during Plate A incubation. Add 270 µL growth medium to each well. After incubation transfer 30 µL from each well of Plate A to the corresponding well in Plate B.



6. Measure absorption at 600 nm ± 20 nm.

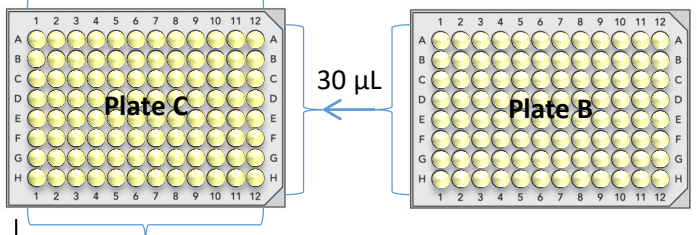
Incubate Plate B at 37°C for 2 hours

7. Measure absorption at 600 nm ± 20 nm.

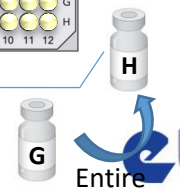


Add 120 µL to all wells

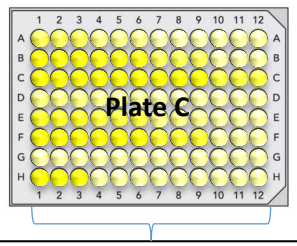
8. Prepare Plate C during Plate B incubation. Add 120 µL reagent I to each well. After incubation transfer 30 µL from each well of Plate B to the corresponding well in Plate C. Add 30 µL chromogen to each well.



Add 30 µL to all wells



Incubate Plate C at 37°C for 30 minutes



10. Measure absorption at 420 nm ± 20 nm.

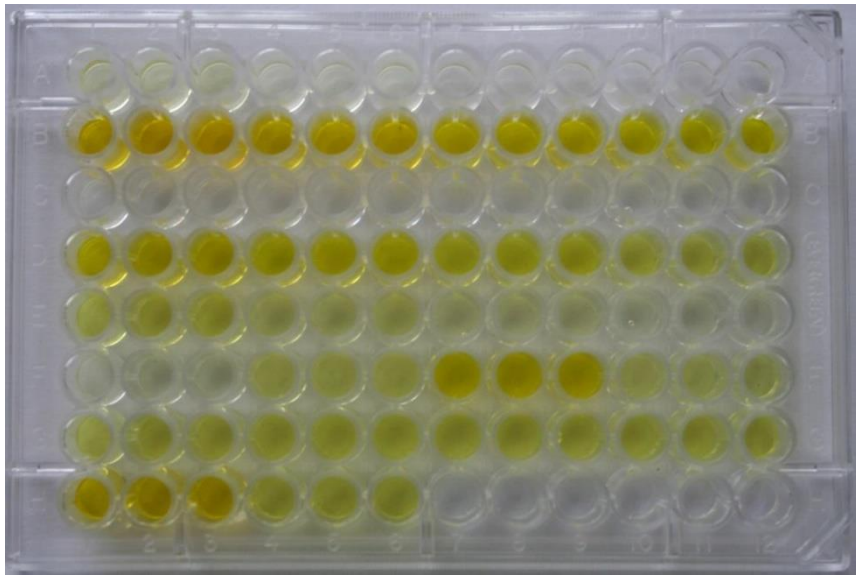
9. Add 120 µL reagent L to all wells

11. Analyze results using EBPI's bioinformatics spreadsheet.

ebpi environmental bio-detection products inc.

UMU-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
- Procedure follows ISO 13829 guidelines to allow comparison of results between laboratories
- Small sample volumes are employed (360 µL).
- UMU-ChromoTest™ is rapid, test is done in one day.
- 6 samples can be run on each plate in triplicate with 4 dilutions.



Bioactivation (S9)

- Many mutagens 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 mutagen 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

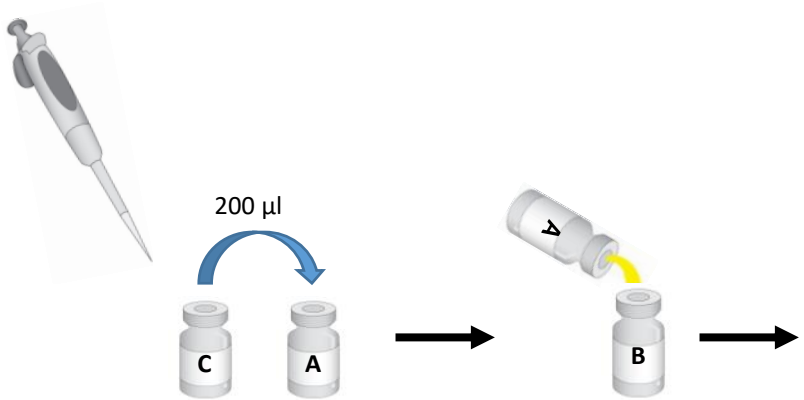
S9 Kit Contents

S9A: $MgCl_2$ + KCl solution (1 unit)
S9B: Glucose-6-phosphate solution (1 unit)
S9C: NADP solution (1 unit)
S9D: Phosphate buffer (1 unit)
S9F: Lyophilized S9 fraction

Detailed Procedure

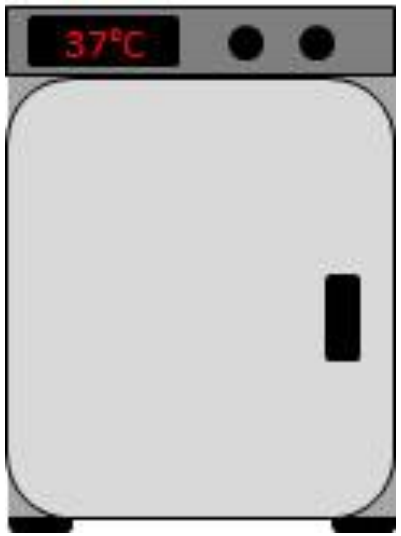
1. Overnight inoculation and initial bacterial growth

- Always use aseptic techniques for all steps in this procedure
- Add 200 µL of 1X glucose (● C) to growth media (● A), mix and immediately transfer to lyophilized bacteria (● B)
- Shake to dissolve and place in incubator overnight at 37 °C with shaking (if possible)
  - aeration will aid bacterial growth.



Pipette 200 µL of **(C)** into the growth media **(A)** bottle

Pour media into lyophilized Bacteria **(B)**

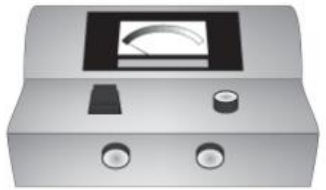


Incubate at 37°C overnight (12-16 hrs)

2. Bacteria dilution and Resuspension

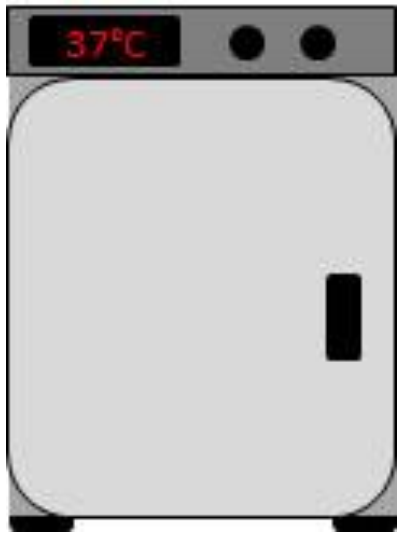
- The next morning observe bacterial vial for turbidity
- If turbidity is seen proceed with initial OD_{600} measurement
- If turbidity is not seen continue growth in incubator
- OD_{600} should be around 0.5 after overnight growth
- Re-inoculate bacteria in 5 mL of freshly prepared growth media (dilute the overnight suspension in half)
- Incubate fresh bacterial suspension at 37 °C for 1.5 hours

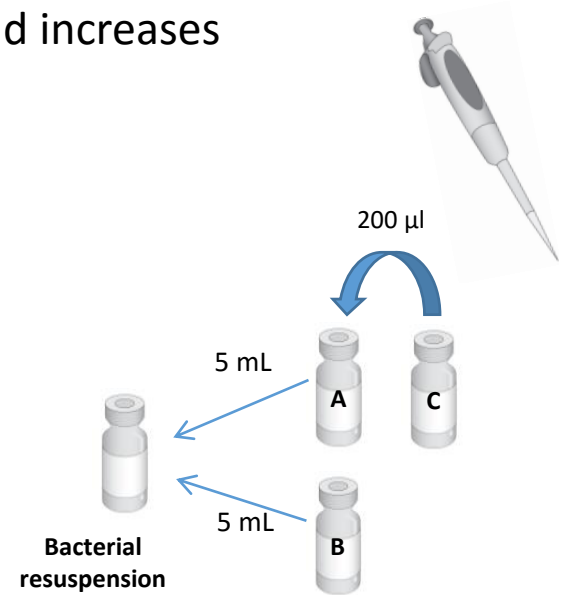
  - Bacterial dilution encourages log growth phase and increases uptake of solution components



2. Measure absorption of 600 nm ± 20 nm light. The absorption (OD) should be ≈ 0.5.

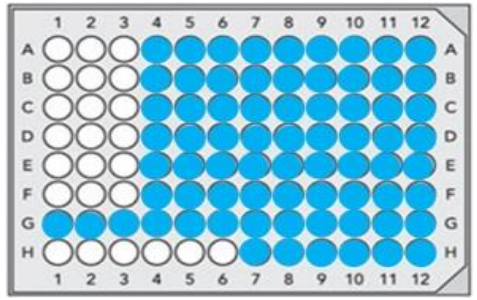
Incubate at 37°C for 1.5 hours



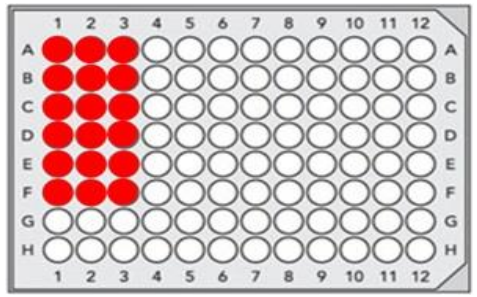


3. Preparation of the Test Plates

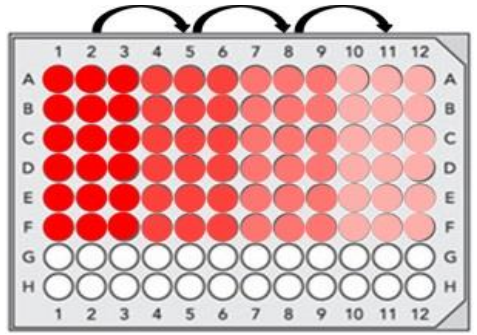
- Add 180 µL of distilled water (MQK) to all wells except **A** to **F, 1-3** and **H 1-6**



- Add 360 µL of test samples to the first three wells in rows **A** through **F**



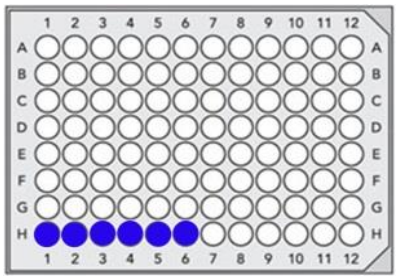
- Prepare a series of 1:2 dilutions of the test samples by transferring 180 µL from wells 1-3 to 4-6 then 7-9 and finally 10-12. Mix each dilution thoroughly using the pipette before transfer. Discard 180 µL from the last three wells **A** to **F**, **10-12**.

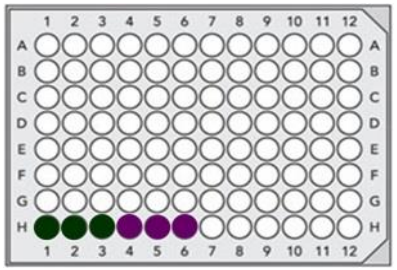


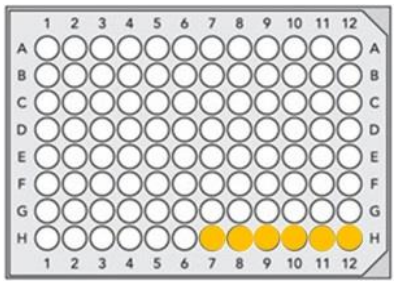
- All wells should have 180 µL **total volume**

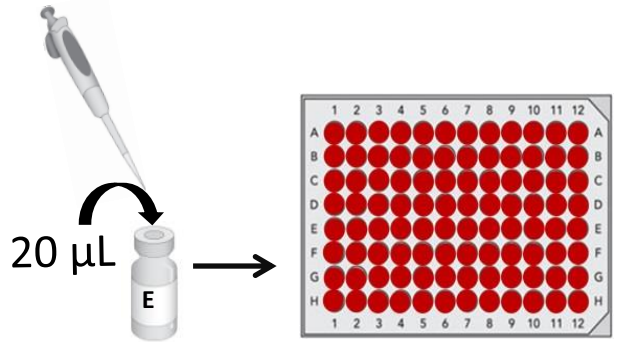
4. Preparation of the Test Plates

- Add **153 µL water (● K)** to the positive-control and solvent-control wells (H1to H6)
- Add **27 µL of a 10% water/DMSO solution (or whatever solvent was used)** as solvent control to wells **H4** to **H6**.
- Add **27 µL of the 4-NQO (● D)** provided as a positive control to well **H1** to **H3**;
- Add 20µL of 10x Growth Media **(● E)** to all wells (A to H, 1 to 12);
- Add **70 µl Growth Media (● A)** to the blanks (H7 to H12) and mix

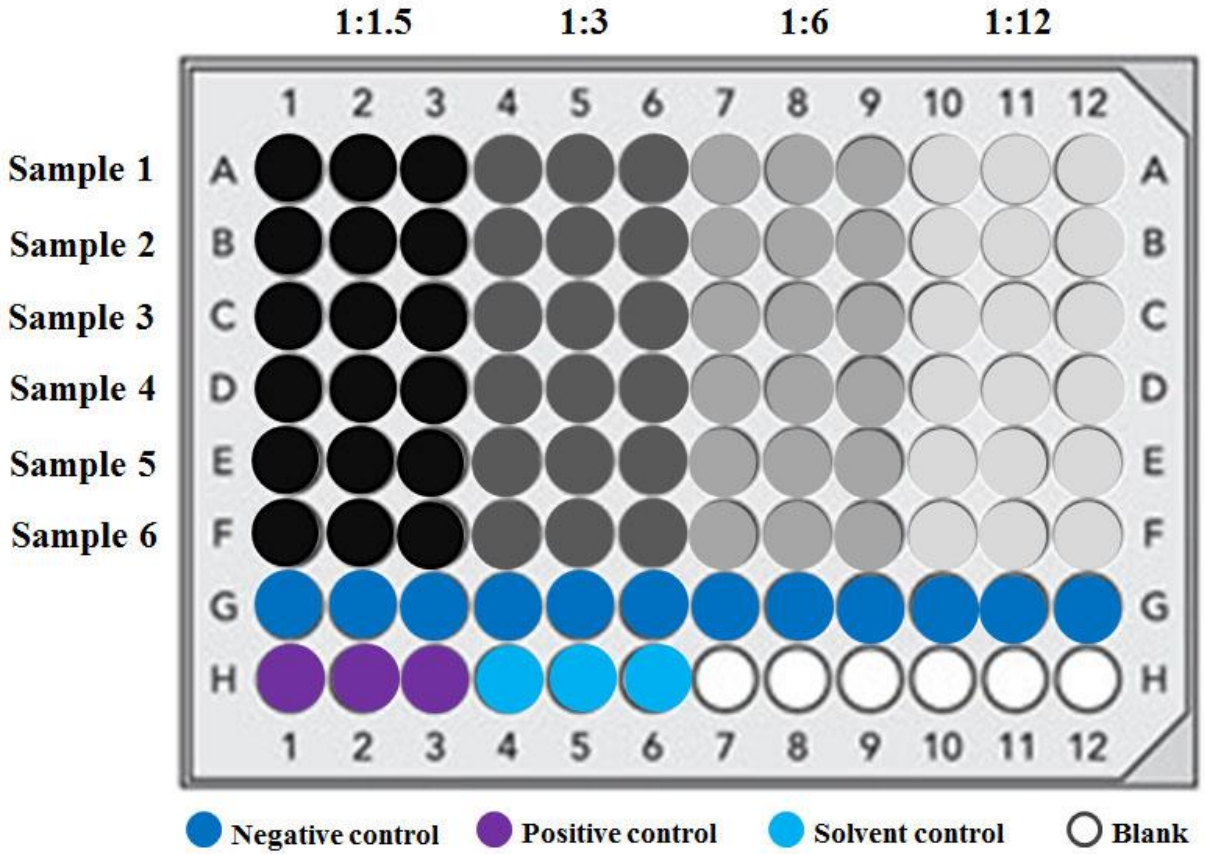








5. Plate Setup

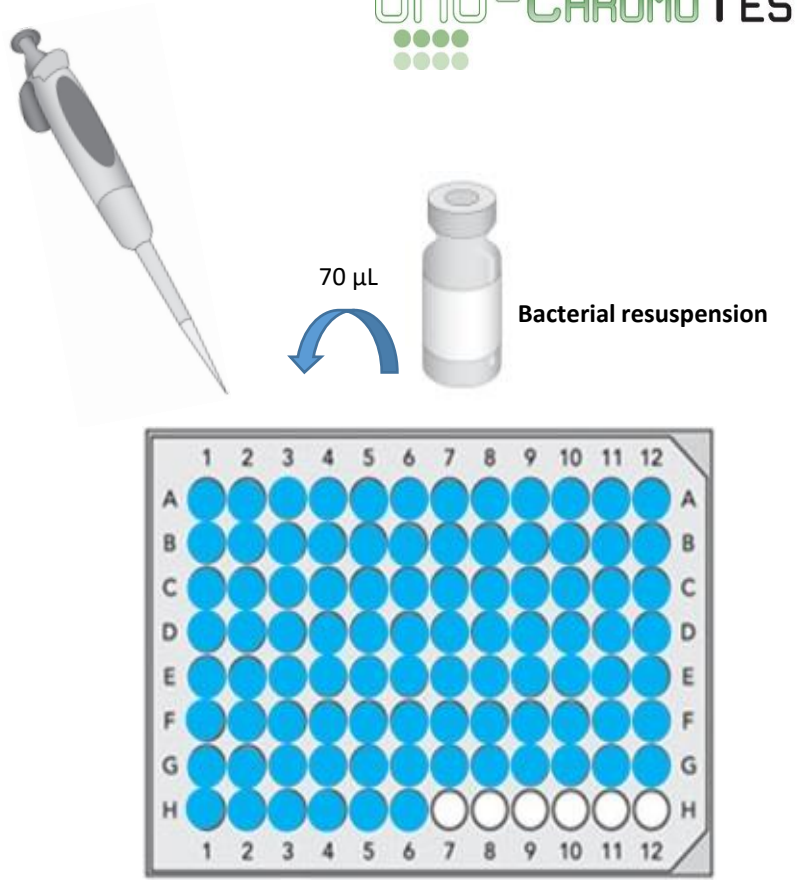


6. Transfer Bacteria into Plate

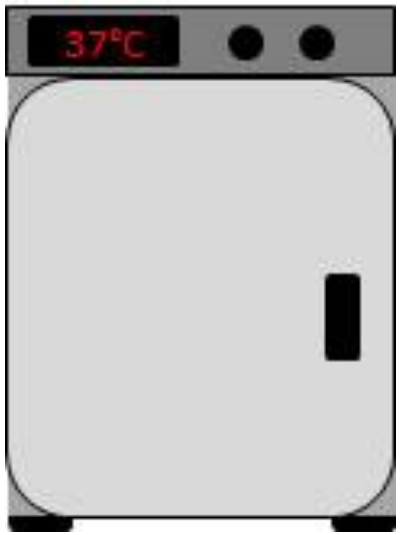
- Once the 1.5 h incubation of **the reinoculated bacteria is complete, and OD_{600} is above 80% of overnight value, pipette 70 µL of the inoculum** to all wells (**A to F, 1 to 12**) and, if concerned with mixing, mix from right to left (lower to higher concentration of sample). Always change pipette tips in between samples

- Pipette **70 µL of inoculum** to the negative-, solvent- and positive-control wells (**G, 1 to 12, H 1 to 6**) and mix. **NO BACTERIA IS ADDED TO ROW H WELLS 7-12**

- **Incubate the plate at 37 °C for 2 h**

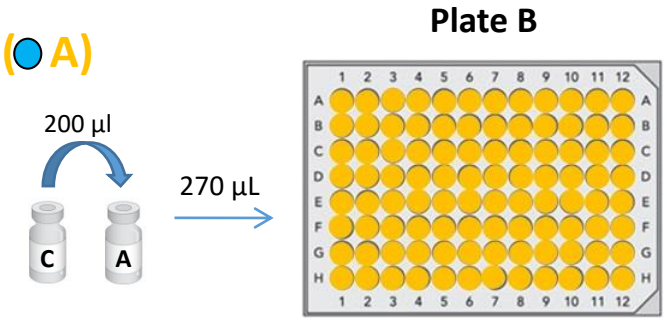


Incubate at 37°C for 2 hours

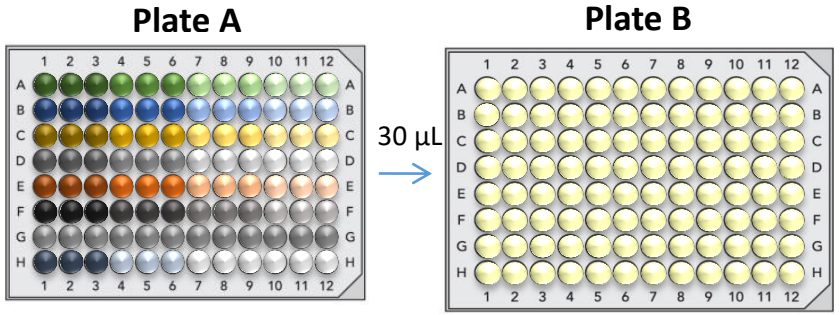


7. Preparation and Incubation of **Plate B**

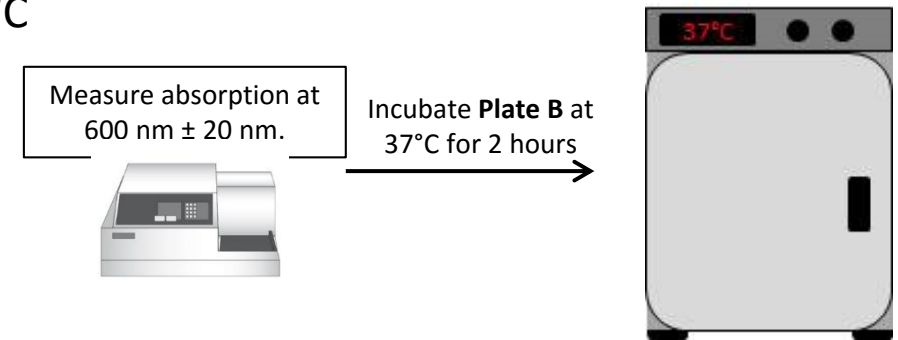
- Toward the end of the incubation time for Plate **A**, add 270 µL of **Growth Media (A) containing 1X Glucose (C)** to each well of a new microplate B and place in an incubator to warm media to 37 °C. Use lids to avoid evaporation.



- Pipette **30 µL from each well of microplate A** into the corresponding well of microplate **B** (performing a tenfold dilution)



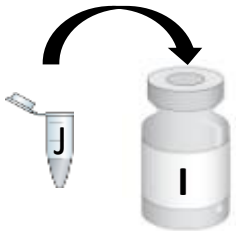
- Measure and record the absorbance of Plate **B** (600 nm ± 20 nm) with a microplate reader before incubation in order to calculate bacterial growth. Incubate microplate **B** for 2 h at 37 °C



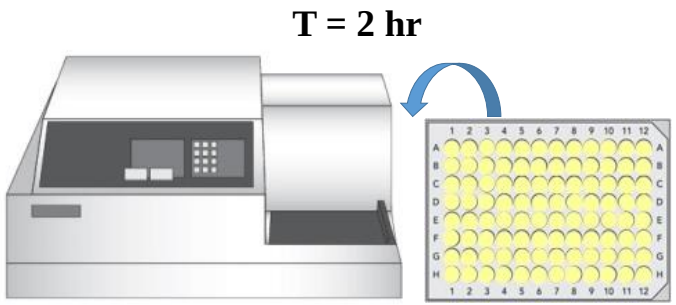
8. Preparation of Plate C

- Toward the end of the incubation of Plate **B**, pour the Phosphate Buffer **(G)** into the amber vial containing the ONPG powder **(H)**. Shake until the ONPG powder is completely dissolved (5 to 10 minutes). Store in the dark at room temperature until use

- At this time you will also need to bring the B-Buffer **(I)** to room temperature in order to dissolve any crystals which may have formed. Once solid has redissolved, add 35 μL of 2-mercaptoethanol **(J)** to the B-Buffer **(I)**



- Measure and record the bacterial growth in Plate B (600 nm ± 20 nm) with a microplate reader after 2 h incubation in order to calculate the growth factor



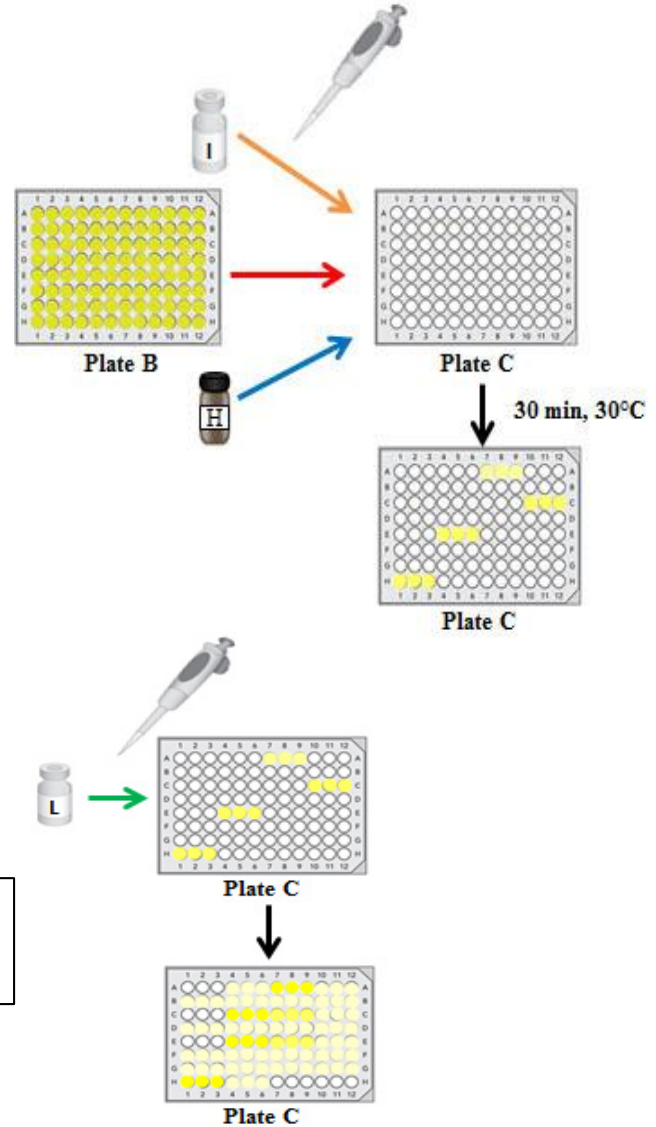
9. Colour Development and Incubation of Plate C

- Dispense **120 µL B-buffer (● I)** in each well of a new microplate **C**. Adjust the temperature to 37 ±1°C in an incubator. Transfer **30 µL from each well of microplate B** (from right to left, i.e. from lower to higher concentration) in the corresponding wells of microplate **C**. Immediately add **30 µL ONPG solution** and mix

- Place the microplate immediately into the incubator for 30 min. Visually inspect plate for colour development. If some yellow colour has developed add **120 µL of Stop Solution (● L)** to each well. If you wish to incubate the final plate longer, keep in mind that adding the Stop Solution increases colour development and may aid in resolution.

- Measure the absorption of the solution in each well at (420 ± 20) nm with a microplate reader.

- As a final step, decontaminate all microplates by autoclaving



Measure absorption at 420 nm ± 20 nm.

Data Interpretation

Genotoxicity is Assessed by Calculating the UMU Induction Ratio (UMUIR) for each well.

To calculate UMU IR two values are required

The growth factor (measures bacterial growth using OD600 after 2 h incubation of PLATE B)

Induction ratio (β-galactosidase activity of bacteria in the exposure wells)

Growth Factor

$$G = \frac{A_{600,S} - A_{600,B}}{A_{600,N} - A_{600,B}}$$

Where $A_{600,S}$ is the absorbance of the sample S at 600 nm

$A_{600,B}$ is the absorbance of the blank at 600 nm

$A_{600,N}$ is the absorbance of the negative control at 600 nm

Important Considerations for Growth Factor:

For IR validity, the growth ratio must be greater than 0.5.

The expected value is between 0.5 and 1, as bacteria will grow less rapidly in a genotoxic environment.

If the growth factor is below 0.5, insufficient growth has occured and results are invalid.

Data Interpretation

- **β-galactosidase activity (relative units)**

- **Us=** $\frac{A_{420,S} - A_{420,B}}{A_{420,N} - A_{420,B}}$

Where $A_{420,S}$ is the absorbance of the sample S at 420 nm

$A_{420,B}$ is the absorbance of the blank at 420 nm

$A_{420,N}$ is the absorbance of the negative control at 420 nm

Important Considerations for β-galactosidase activity

The β-galactosidase activity measures how much ONPG was broken down to create a yellow colour in the sample, as compared to the negative control. Since samples that induce a genotoxic response result in more β-galactosidase being produced, negative control wells should have the lowest reading. If readings at the highest tested concentrations are low, it may indicate acute toxicity.

UMU IR

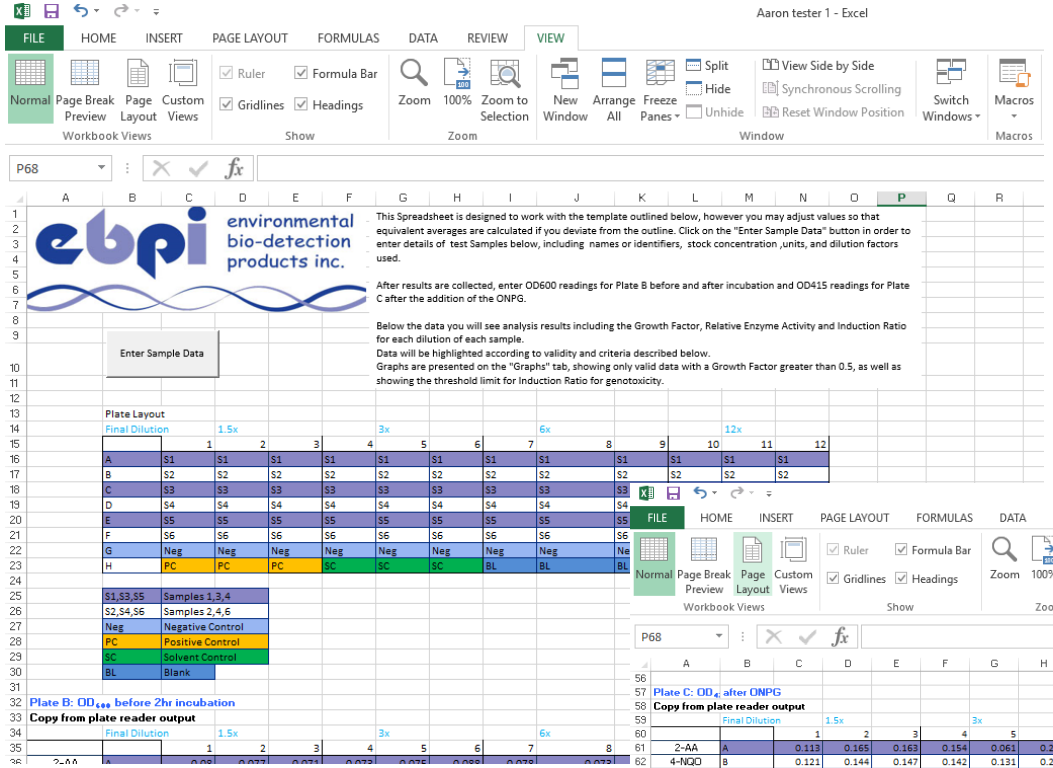
- **Induction Ratio**

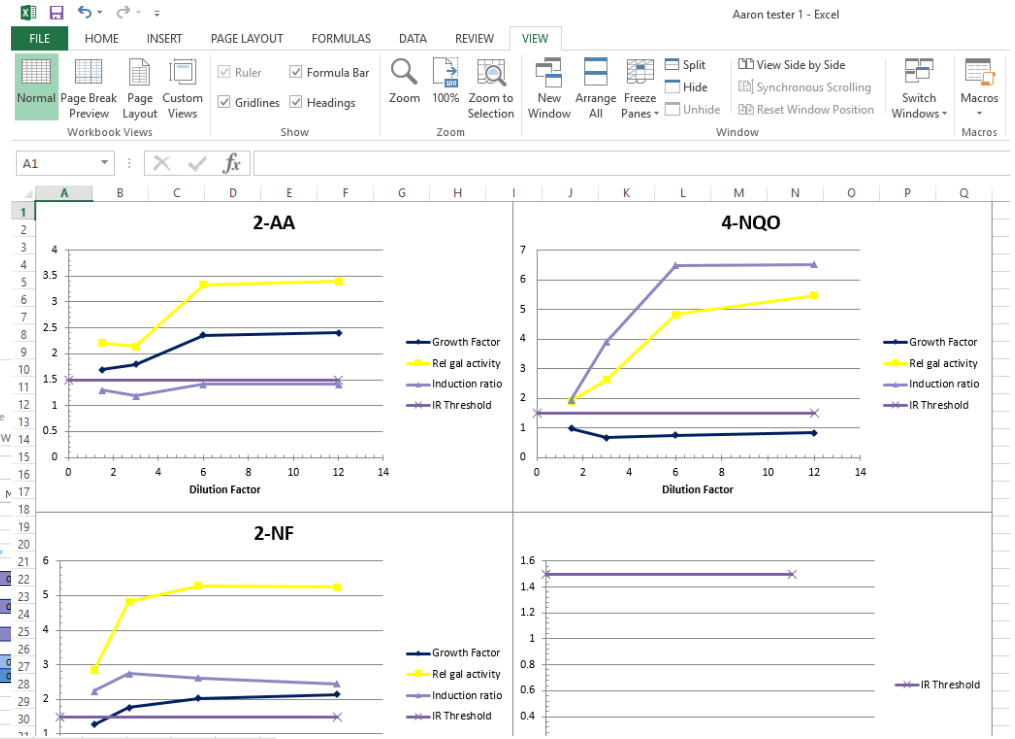
$$\mathbf{I_R} = \frac{\mathbf{1}}{\mathbf{G}} \times \mathbf{U_S}$$

- **Important Considerations for Induction Ratio**

- In order to consider a test sample genotoxic, the induction ratio must be greater than 1.5, and the growth factor greater than 0.5.
- Validation of the test is presumed if the positive controls reach an induction ratio of greater than 2.
- A few values of interest are the lowest effective dilution, which is the lowest dilution of the dilution series with an IR < 1.5, as well as the highest ineffective concentration which is the highest concentration within the dilution series with an IR < 1.5.
- In order to facilitate the analysis of experimental results, an Excel template is available from EBPI, which allows for entry of all absorbance readings, and will calculate growth factors, β-galactosidase activities and induction ratios for each sample.

UMU-Chromotest EXCEL Spreadsheet





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UMU-ChromoTest™

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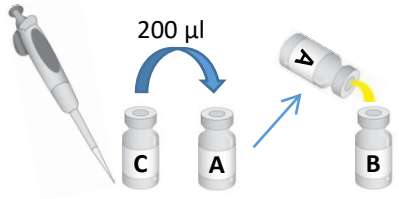

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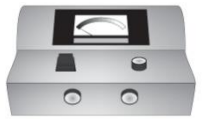
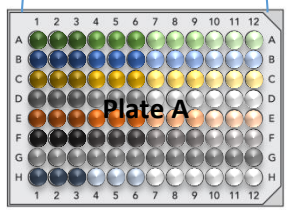


3. Re-inoculate 5 mL of bacterial suspension in new growth medium.

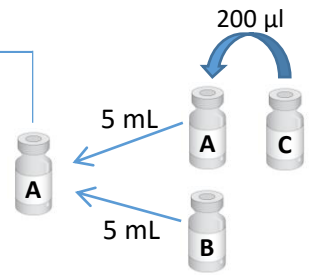
Incubate Plate A at 37°C for 2 hours

4. Prepare Plate A during bacterial suspension incubation by performing serial dilutions for all samples. Plate A will also include all controls (positive, negative, solvent) and blanks. After incubation, dispense 70 µL of bacterial suspension to all appropriate wells.

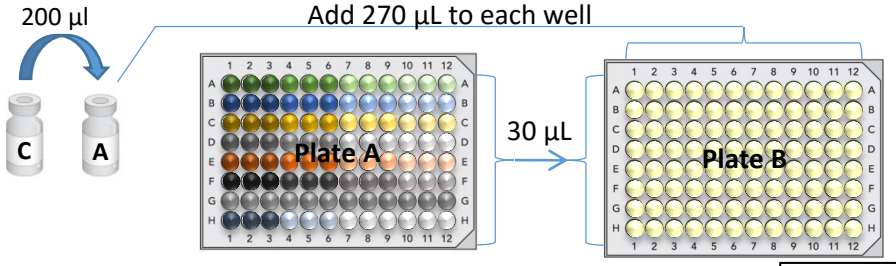
Add 70 µL to all appropriate wells



Incubate at 37°C for 1.5 hours



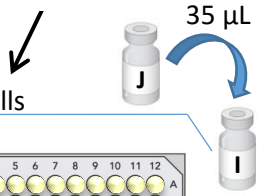
5. Prepare Plate B during Plate A incubation. Add 270 µL growth medium to each well. After incubation transfer 30 µL from each well of Plate A to the corresponding well in Plate B.



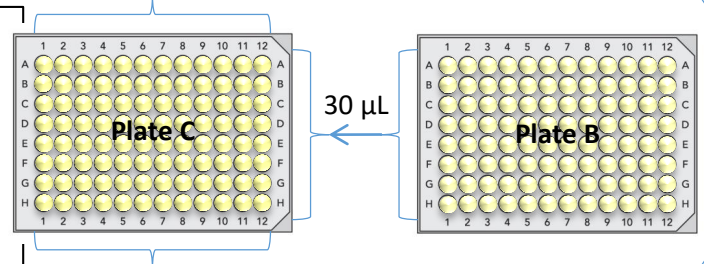
6. Measure absorption at 600 nm ± 20 nm.

Incubate Plate B at 37°C for 2 hours

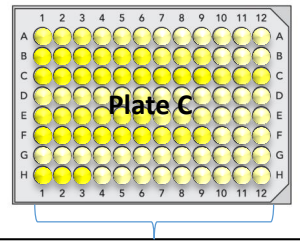
7. Measure absorption at 600 nm ± 20 nm.



8. Prepare Plate C during Plate B incubation. Add 120 µL reagent I to each well. After incubation transfer 30 µL from each well of Plate B to the corresponding well in Plate C. Add 30 µL chromogen to each well.



Incubate Plate C at 37°C for 30 minutes



9. Add 120 µL reagent L to all wells

10. Measure absorption at 420 nm ± 20 nm.

11. Analyze results using EBPI's bioinformatics spreadsheet.

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UMU-Chromotest™ Kit Options

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  - Bioactivate procarcinogens without S9
  - Increased sensitivity for PAH and nitroaromatic carcinogens
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