TOXI-CHROMOTEST™

Users Guide

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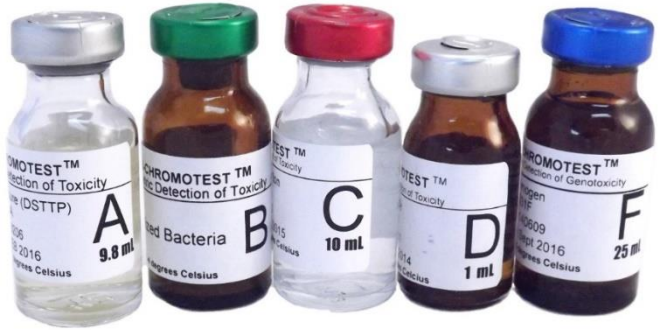
Kit Contents:

Standard Reagent List

- A: Rehydration Solution (3 Sets)
- B: Toxi-ChromoTest Lyophilized Bacteria (3 Sets)
- C: Reaction Mixture (3 Sets)
- D: MgCl2
- F: Chromogenic Substrate (3 Sets)
- G: Diluent (3 Sets)



Disposable list

- 96-well plate (3 units)
- A disposable biohazard bag

Required Equipment

- Micropipette using disposable sterile tips in the range of 100 µl to 1000 µl
- Vortexer (optional)
- Microplate Reader (optional)
- A 37˚C incubator

TOXI-CHROMOTEST™

Applications:

- Testing of industrial effluents for presence of possible toxic compounds.
- Routine monitoring of waste water effluent for heavy metal toxicity.
- Screening of recycled potable water supplies for presence of priority pollutants and toxins.
- Evaluating water and soil samples for elevated levels of acute toxins.
- Effective teaching tools for University and College laboratories.

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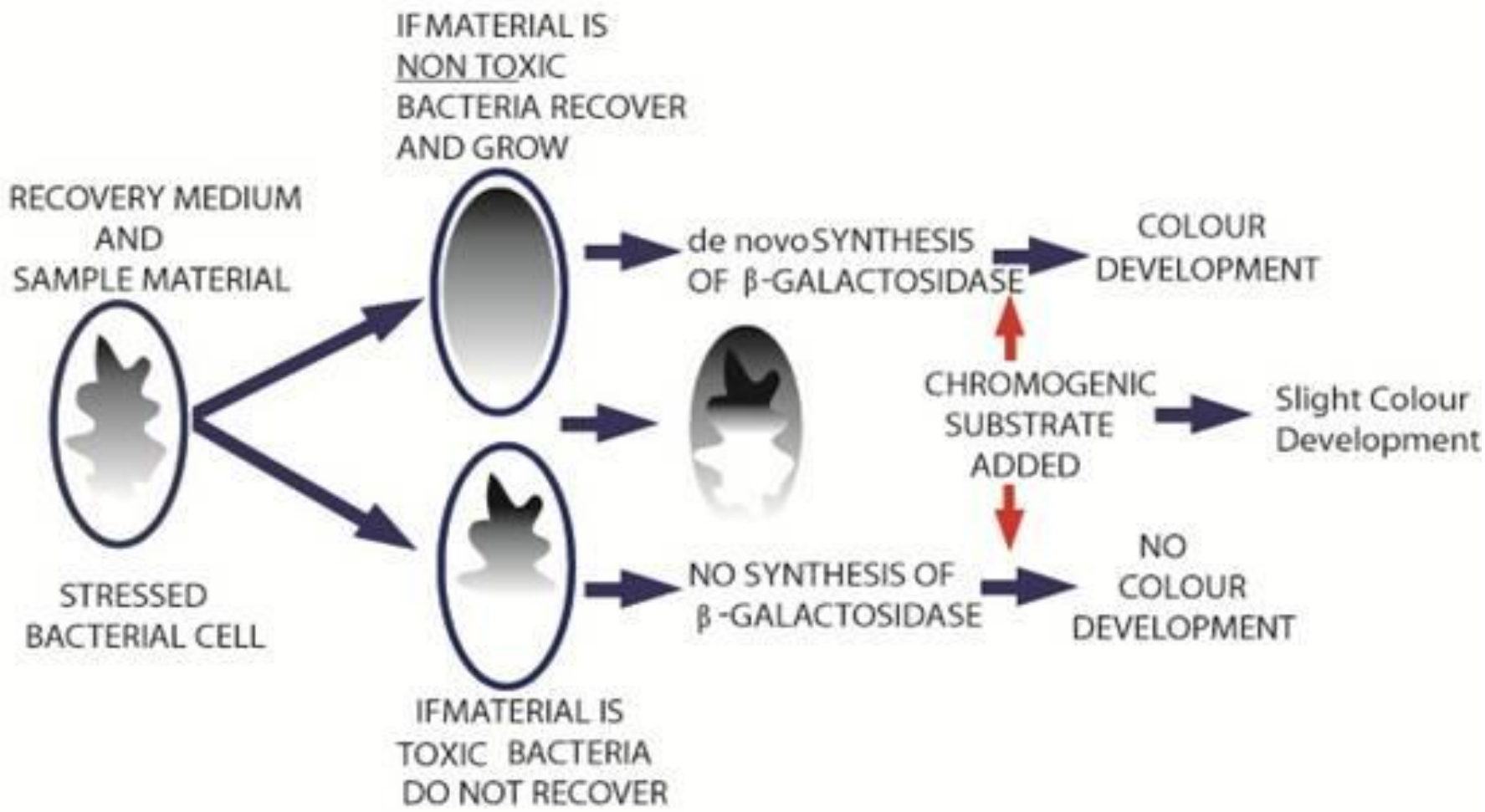
TOXI-CHROMOTEST™



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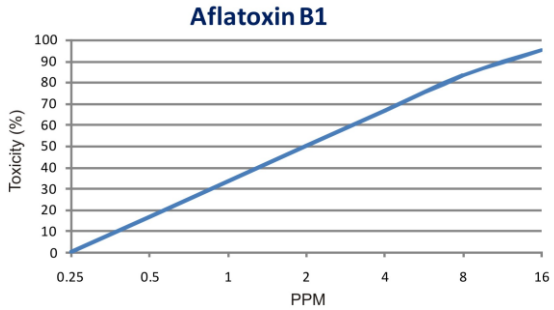
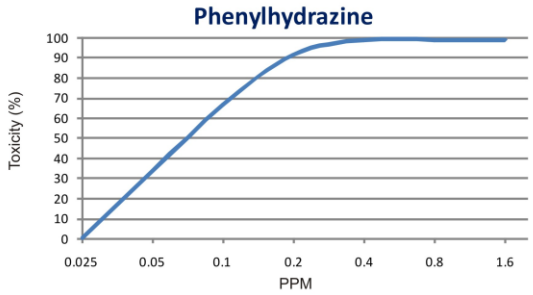
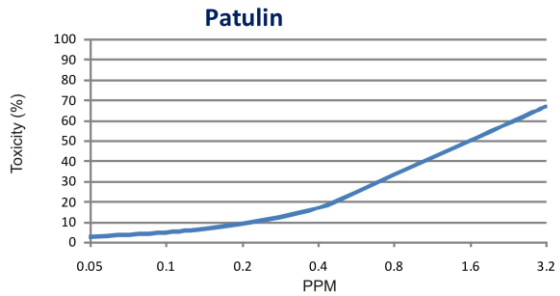
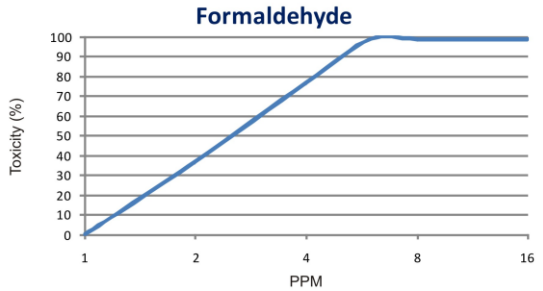
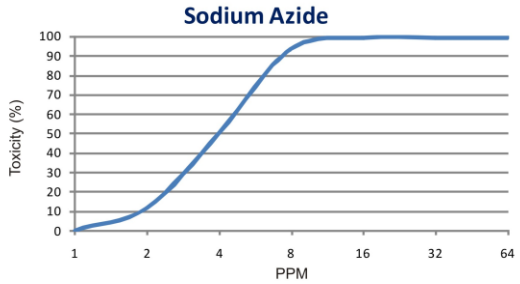
Examples of toxicants tested with the Toxi-ChromoTest bacteria.

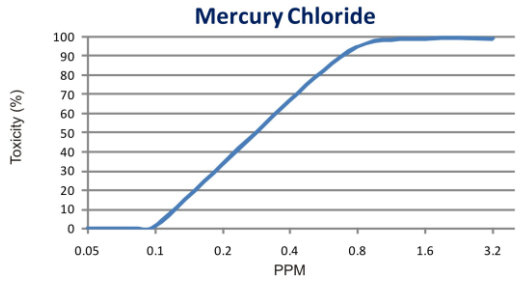


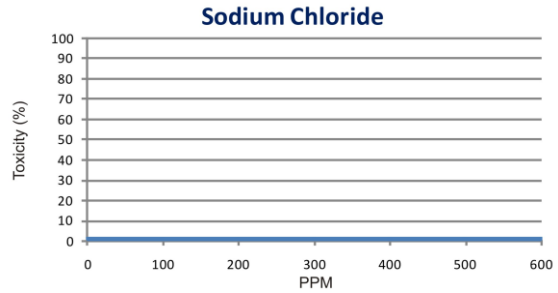






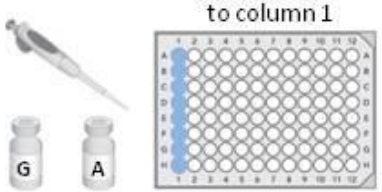




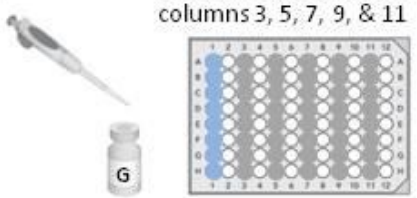


TOXI-CHROMOTEST™

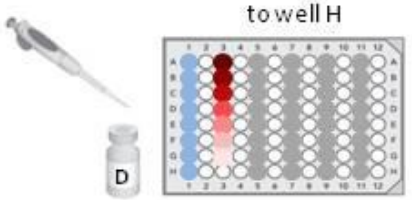
1. Add 100 µL diluent to column 1. Add 100 µL reaction mixture without bacteria to column 1



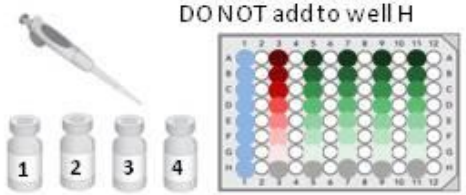
2. Add 100 µL diluent to columns 3, 5, 7, 9, & 11



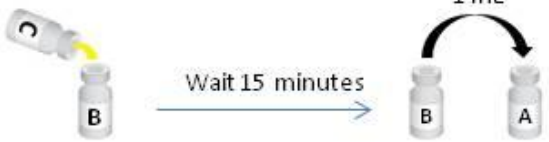
3. Add 200 µL standard toxicant to column 3 well A. Perform a serial dilution down the column. DO NOT add to well H



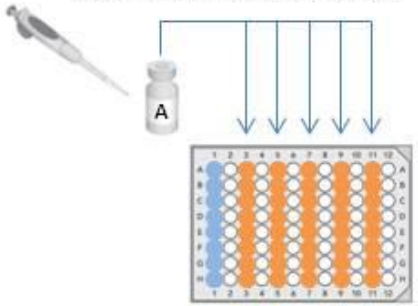
4. Perform serial dilutions down columns 5, 7, 9, & 11 using unknown samples. DO NOT add to well H



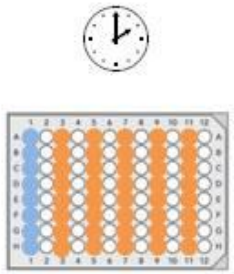
5. Rehydrate bacteria



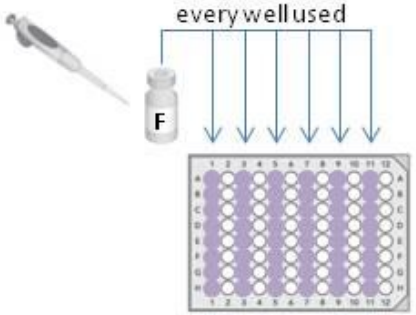
6. Add 100 µL bacterial suspension to all wells in columns 3, 5, 7, 9, &11



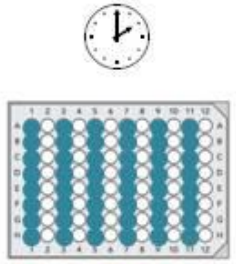
7. Incubate at 37 ºC for 90 minutes



8. Add 100 µL chromogen to every well used



9. Incubate at 37 ºC for 30 minutes or until blue colour appears



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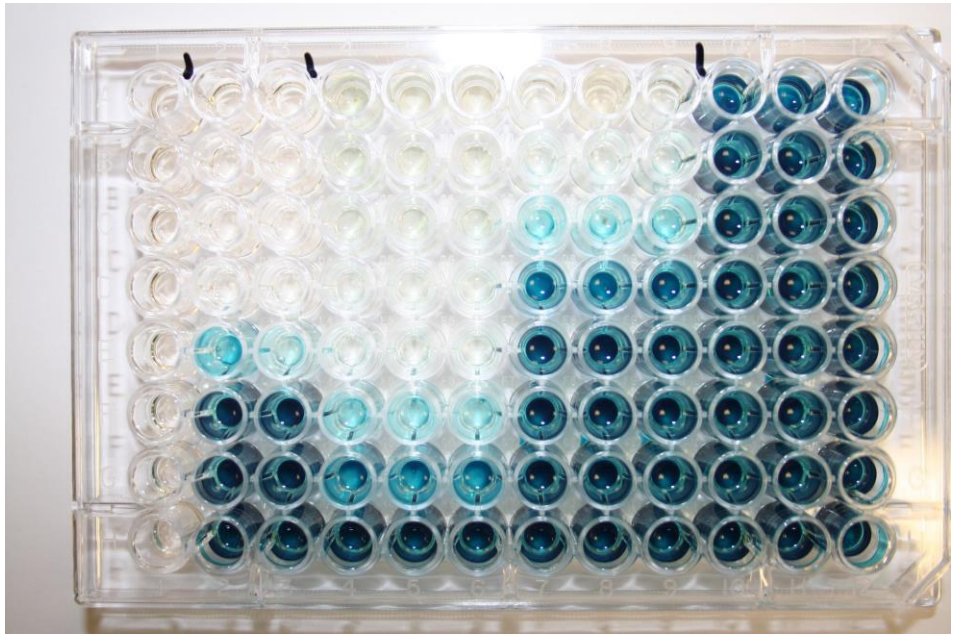
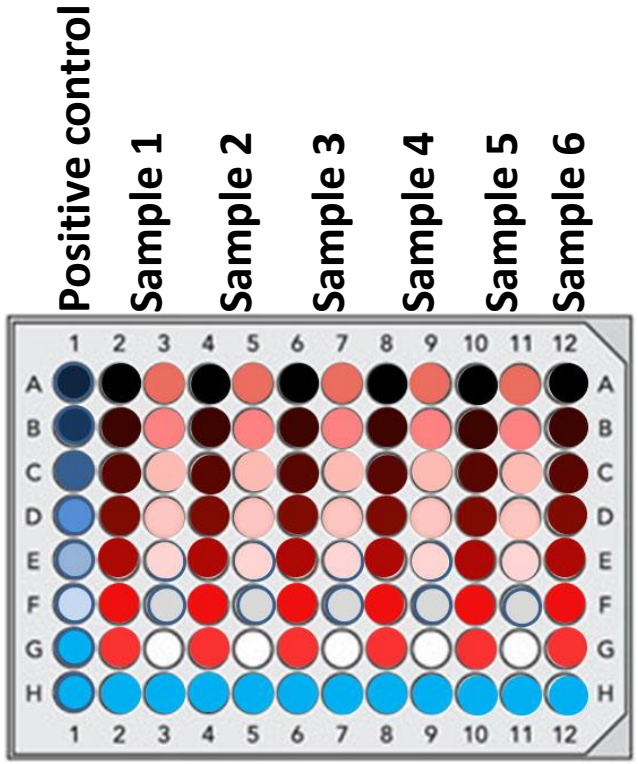
TOXI-ChromoTest™ Advantages

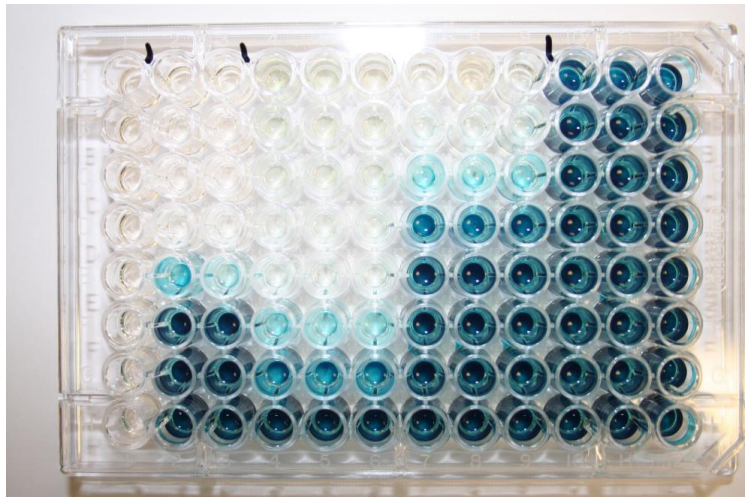
- Reagents, bacteria and other consumable components are supplied ready-to-use in a non-specialized laboratory
- Assay endpoint is a highly sensitive colorimetric change that is easily interpreted
- Small sample volumes are employed (100 µL).
- TOIX-ChromoTest™ is rapid, test is done in one day.
- Test can be conducted on 3 different days.
- Test allows for 288 endpoints.
- Low cost, minimal equipment required.



Toxi-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

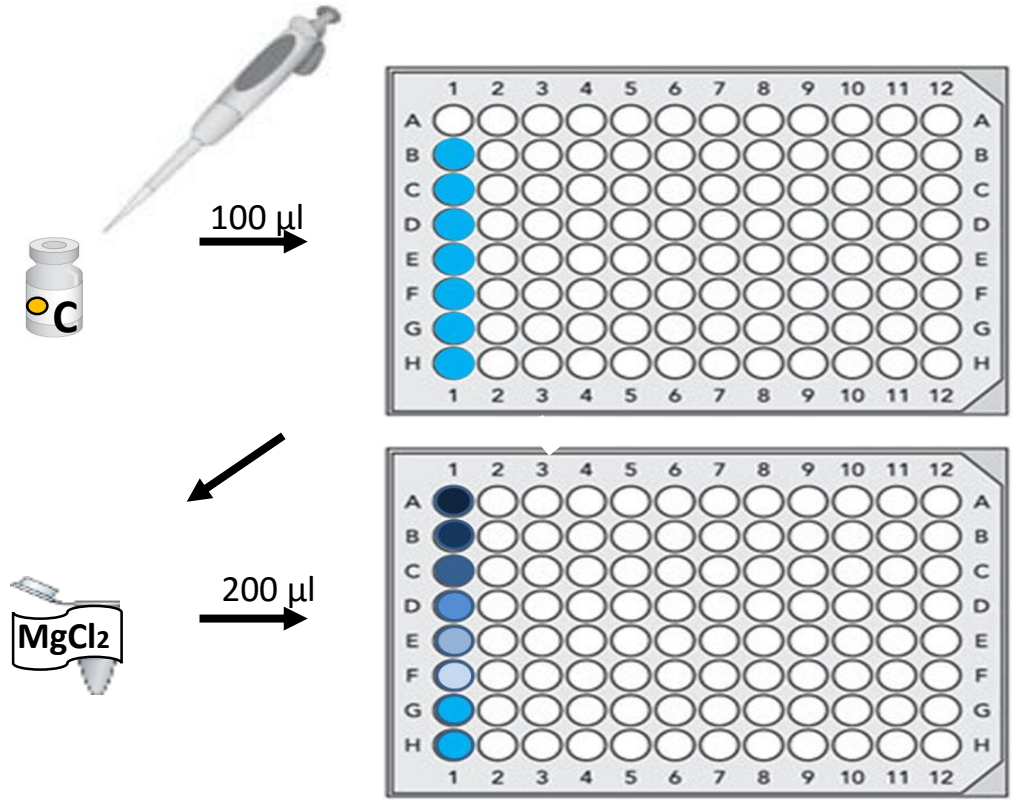
1. Bacterial Growth

- Always use aseptic techniques for all steps in this procedure
- Add TOXI Rehydration solution to bacterial bottle (15 minutes prior to running the assay.

Pour media into lyophilized vial

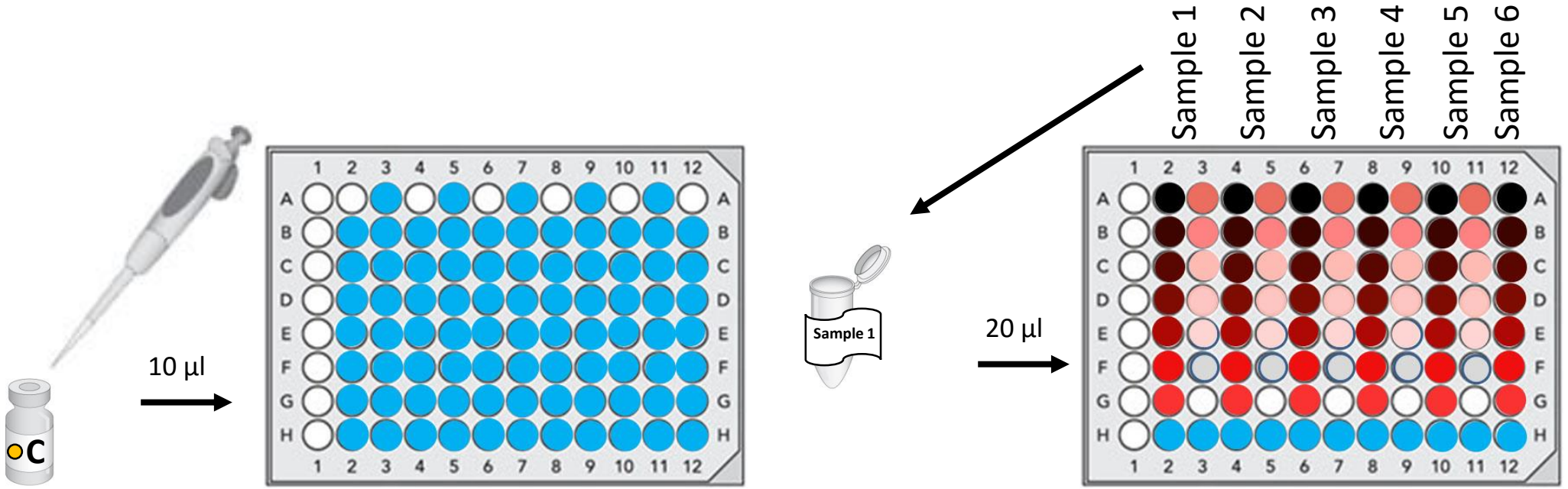
2. Preparation of positive control column

- Add 100 µl of diluent B1-H1
- Add 200 µl of $MgCl_2$ to A1 and perform a serial dilution with column 1.



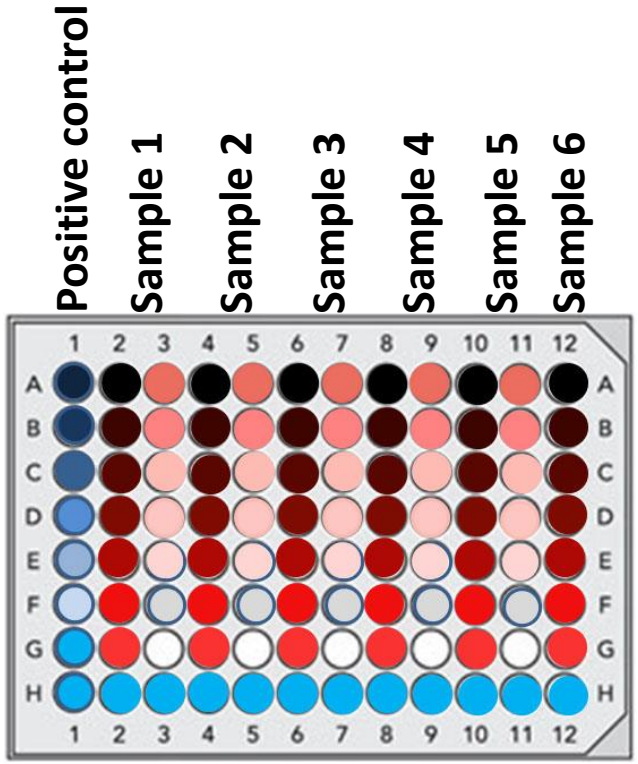
3. Add samples to plate

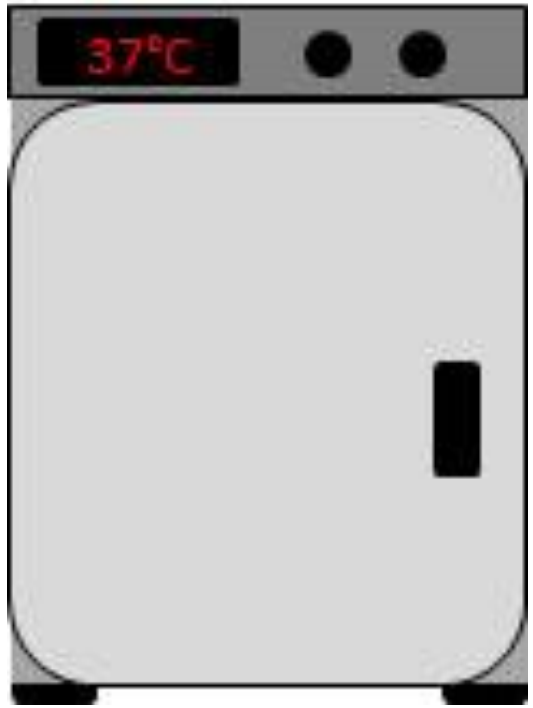
- Add 100 µl of diluent in plate **(except for A2, A4, A6, A8, A10, A12, Column 1)**
- Add 200 µl of samples respectively into wells **(A2, A4, A6, A8, A10, A12)** and follow up with a serial dilution.



4. TOXI incubation

- Incubate plate at 37°C for 90 mins





Incubate at 37°C for 90 minutes

4. Add Chromogenic Substrate

- Add 100ul of the Chromogenic Substrate to each well

5. Incubation

Incubate at 37°C for 30 minutes (or until colour develops)

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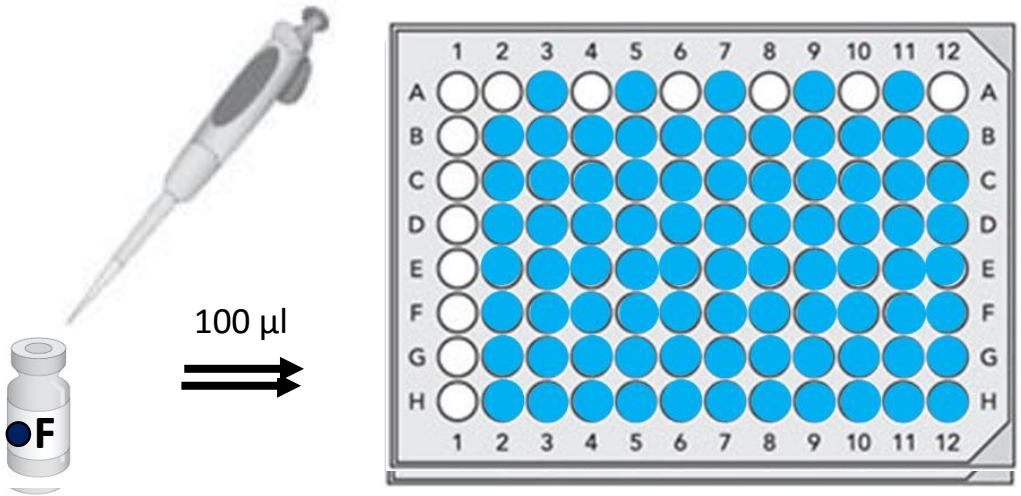

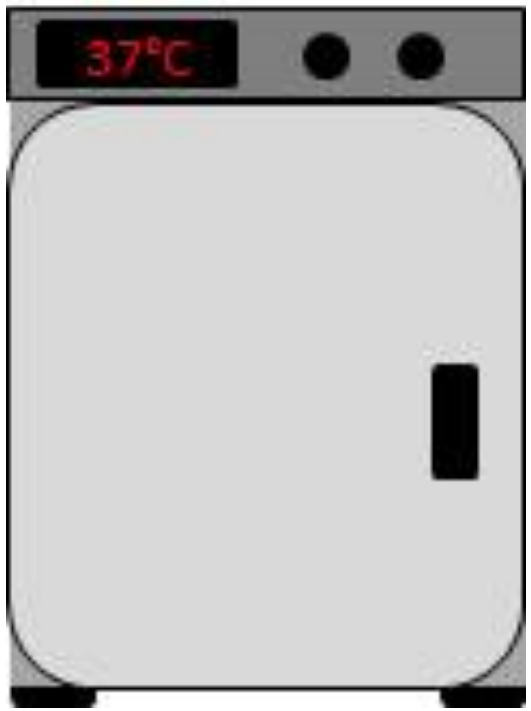

Incubate at 37°C for 30 minutes

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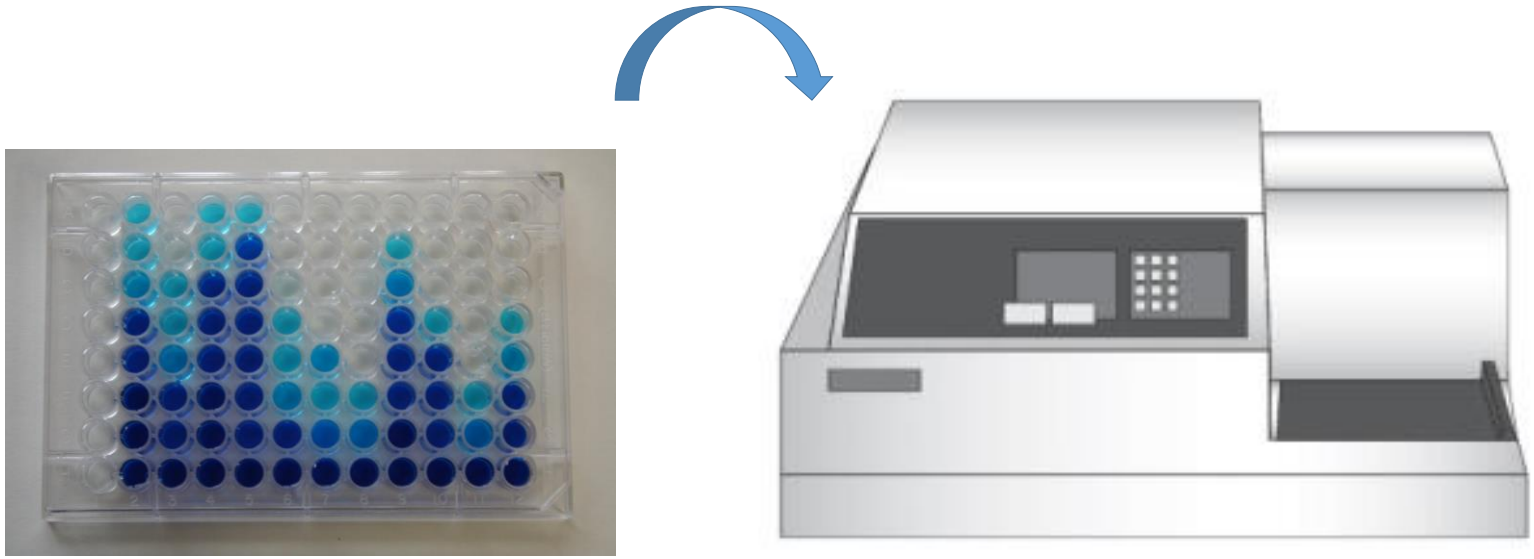
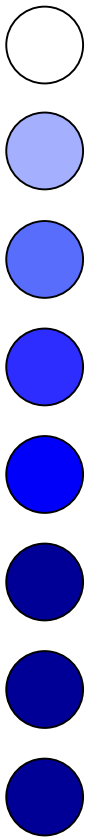
6. Plate reading

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



Analysis of Results



Clear well = 100% Toxicity

10%

20%

40%

60%

0%

0%

Negative Control = 0% Toxicity

- Assign % Toxicity Values to each well by reading visually.
- Calculate Toxicity Factor of each well using the formula

$TF=1-(OD_{615}\ \text{sample}/OD_{615}\ \text{negative control})$

Create dose response curve with your positive control.

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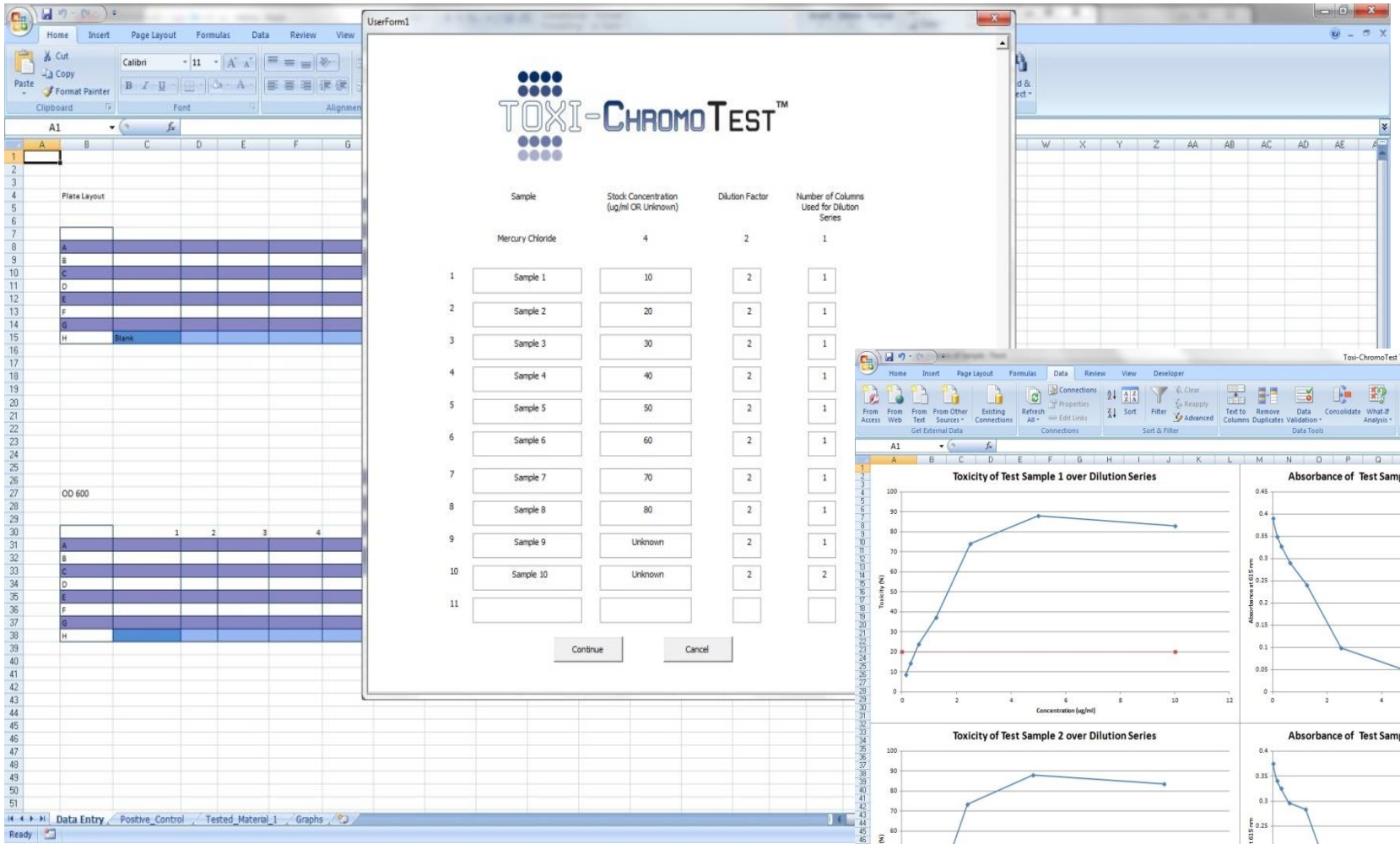
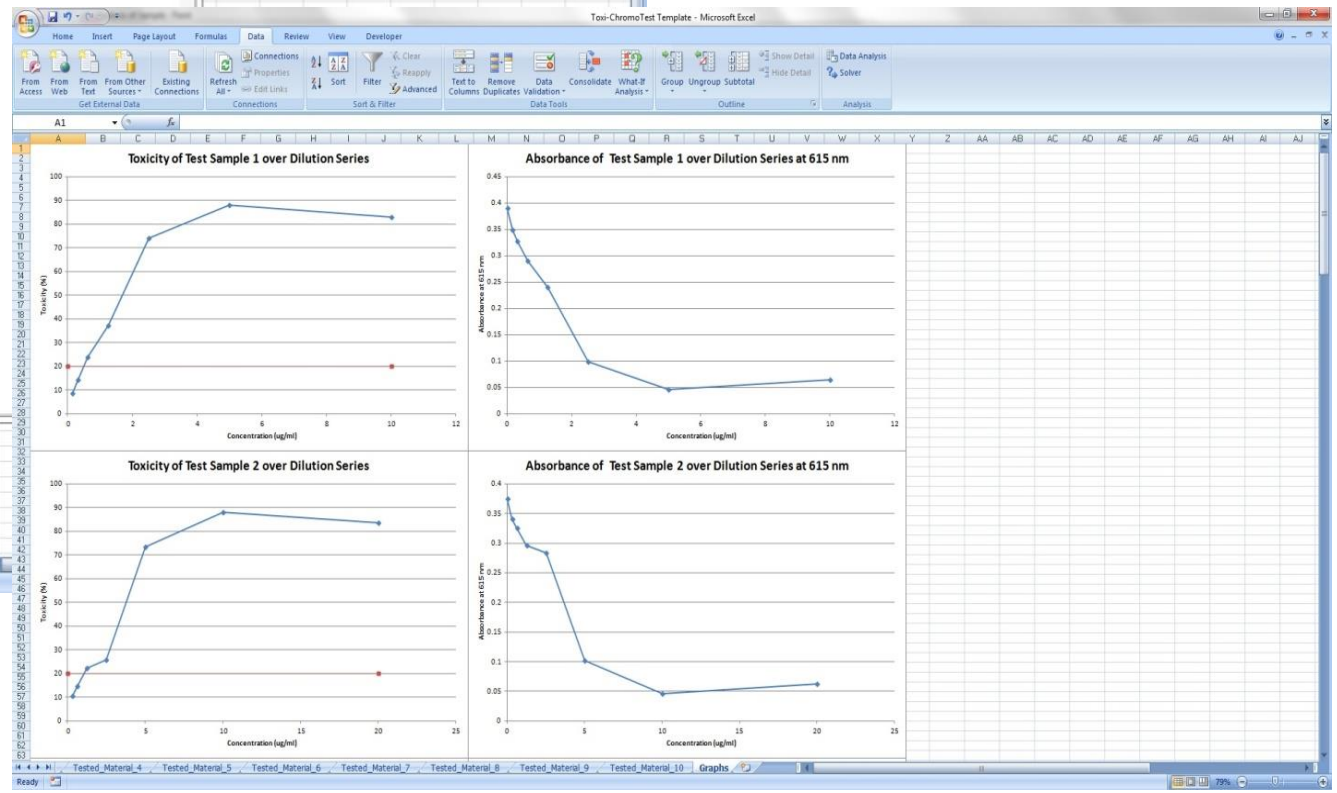
TOXI-CHROMOTEST™





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TOXI-CHROMOTEST™

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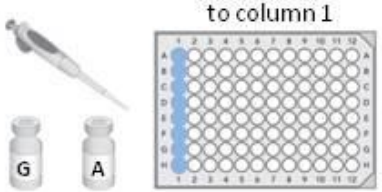
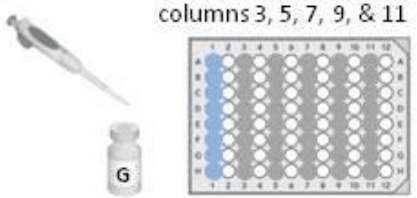


2. Add 100 µL diluent to columns 3, 5, 7, 9, & 11



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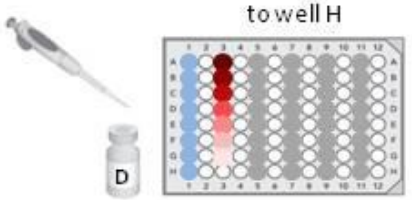
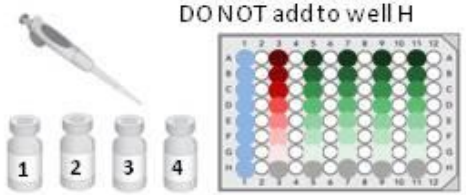
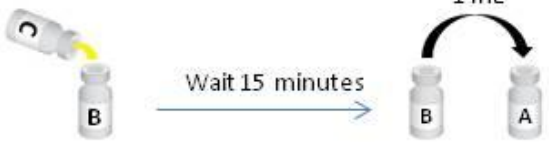


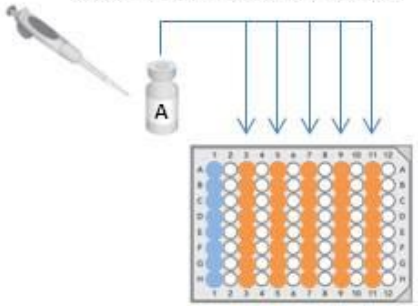
4. Perform serial dilutions down columns 5, 7, 9, & 11 using unknown samples. DO NOT add to well H



5. Rehydrate bacteria



6. Add 100 µL bacterial suspension to all wells in columns 3, 5, 7, 9, &11



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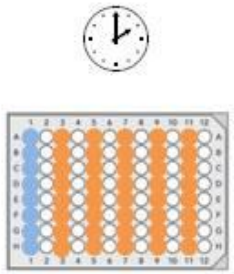
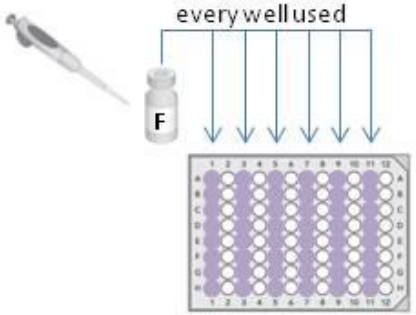


8. Add 100 µL chromogen to every well used



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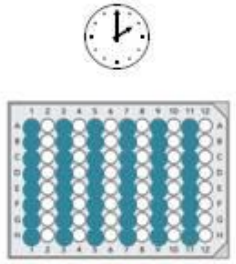

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TOXI-CHROMOTEST™

Kit Options

- Basic Kits
- All kits/reagents can be modified to meet your requirements
- Educational Kits
- Sediment Toxicity Kits

TOXI-CHROMOPAD™

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