Users Guide

ebpi environmental bio-detection products inc.

Applications:

- Testing soils and sediments directly for possible mutagenic compounds.
- Screening of municipal discharges for spill contamination, improper chemical disposal.
- Assessing remediation activities of old industrial sites
- Assessment of soils at spill sites for quality and mutagenicity.
- Evaluating soil and sediment 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.

Kit Contents:

Reagent List

A) Reaction mixture - a cocktail containing an enzymatic inducer and cofactors required for the recovery of test bacteria from their stressed condition. (3 vials)

B) SediTox™ lyophilized bacteria - a highly permeable selected mutant of *E. coli*. (1 vial)

C) Rehydration solution - a solution to rehydrate the lyophilized bacteria. (1 vial)

D) Positive control $HgCl_2$ 1 mL (1 vial)

F) Blue Chromogen (1 vial)

G) SediTox™ diluent 10 mL (4 vials)

SediTox™ Quantitative Calculation kit includes (Add-on kit)

E) Lyophilized chromogenic substrate ONPG. (1 vial)

H) Chromogenic Diluent. (1 vial)

L) Stop solution (1 vial)

Wash Water Bottle 120 mL (1 unit)

Plastics and additional components:

Disposable plastic pipets. (3 pipets)

Disposable test tubes. (25 tubes)

Disposable SPT filter cartridges (25 units)

Sterile 96-well microplate. (1 unit)

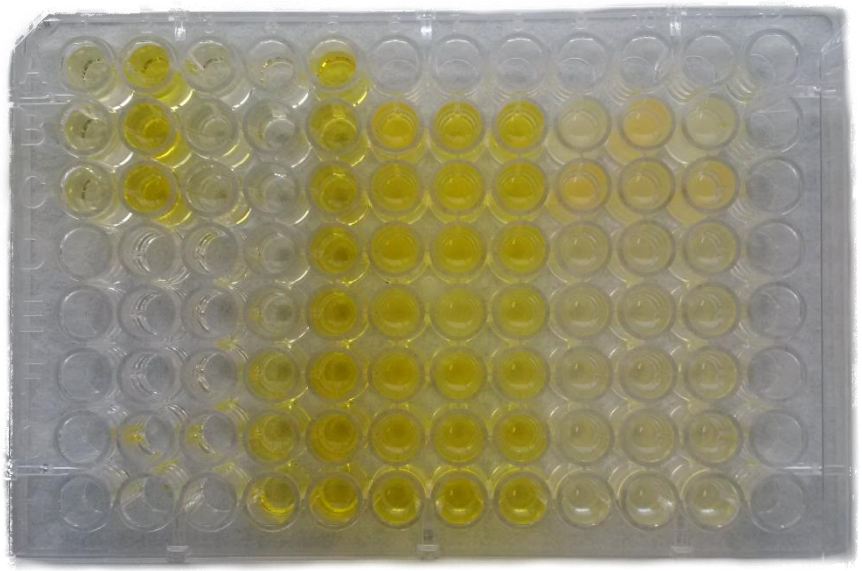
Reference Soil (1 unit)

Disposable 0.45 µm syringe filters (3 units)

Disposable 10 mL syringes (3 units)

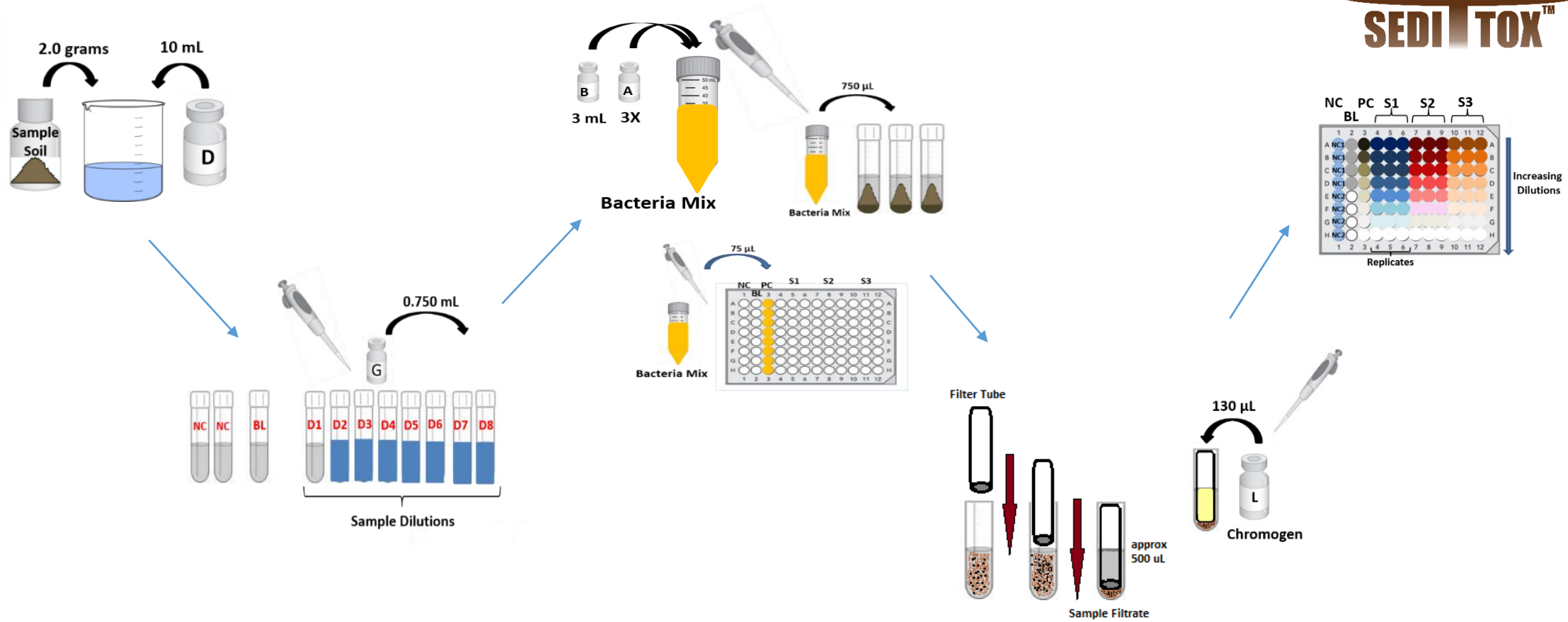
Seditox™ Advantages

- Reagents, bacteria and other consumable components are supplied ready-to-use in a non-specialized laboratory
- Assay endpoints are easy to read colorimetric changes that require no specialized training
- Testing is done directly on soil samples without extraction or filtration mechanisms
- Qualitative screening applications and quantitative laboratory applications
- First direct genotoxicity assessment assay available!
- Less sample is required for testing. Small sample masses produce great results.



Sample Seditox™ result plate

Seditox™ Procedure Overview



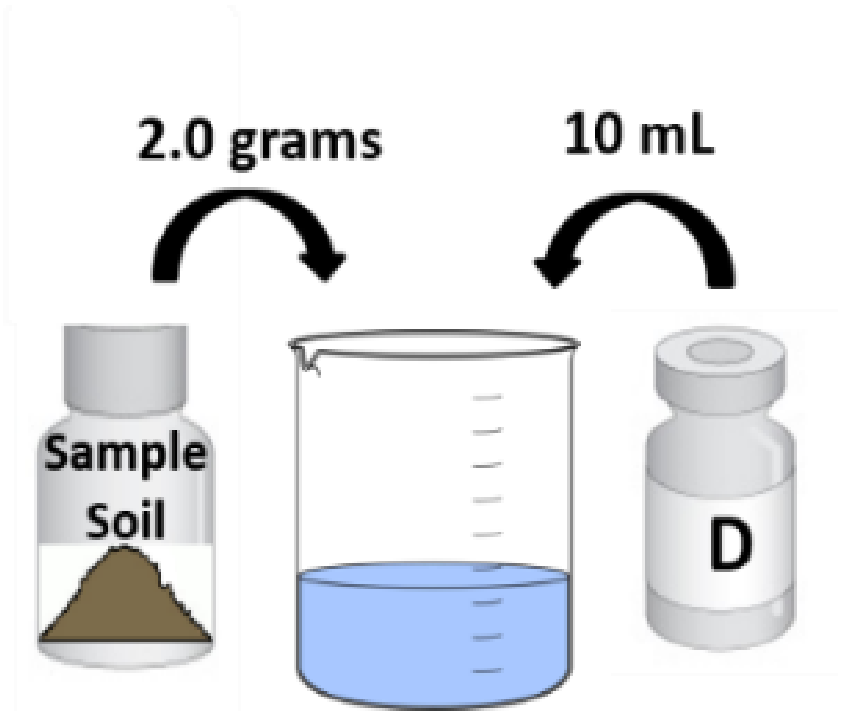
Note: Prior to using our test kits, we highly recommend the development of individual outlines that are representative of the respective experiment. This outline is only provided as a guideline for one possible method

The image above is an example of a test that utilizes one sample with eight distinct dilutions with triplicate replicates.

Detailed Procedure

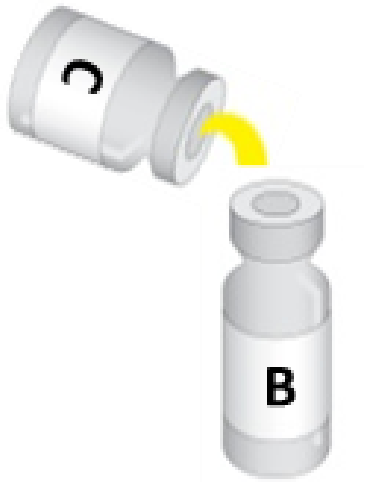
1. Sample Preparation

- Prepare concentrated sample by mixing 2.0 grams of dried particulate with 10 mL Sedi-Tox diluent
- Mix thoroughly for 10 minutes at room temperature to homogenize the mixture .



2. Bacterial Rehydration

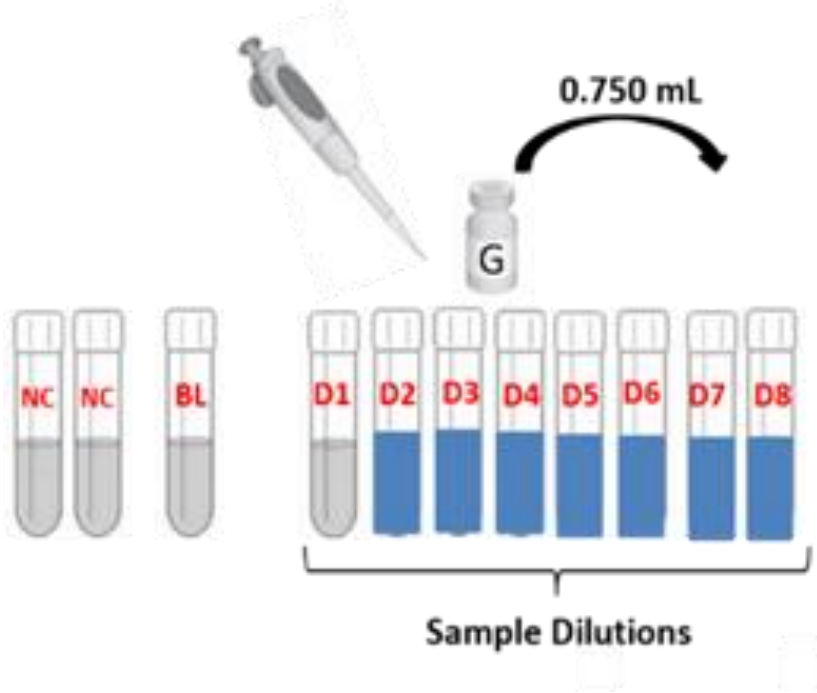
- Rehydrate the Sedi-Tox bacteria by pouring the entire contents of bottle C into the lyophilized bacteria (Bottle B)
- Let the bacteria rehydrate for 15 minutes at room temperature prior to use
- While the bacteria are rehydrating, prepare the sample dilutions

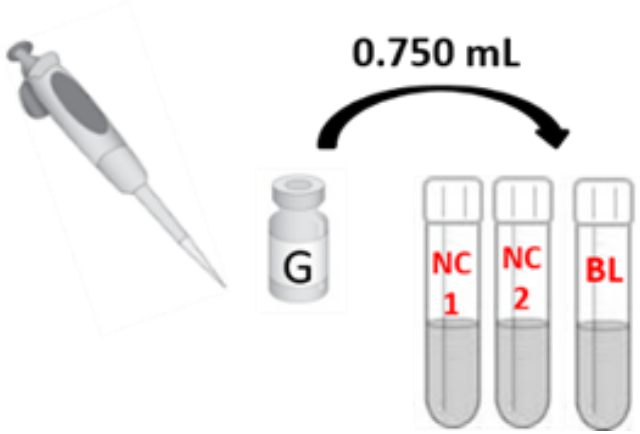


Leave for 15 minutes

3. Prepare Sample Dilutions

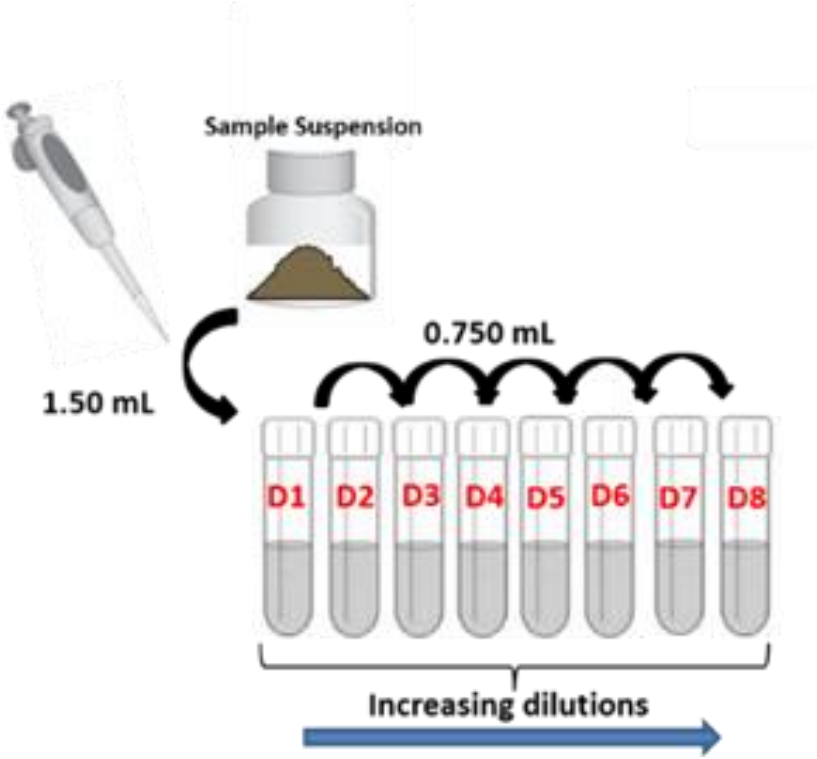
- Prepare test tubes for dilutions by pipetting 0.750 mL of Sedi-Tox sample diluent into labelled tubes
- Pipette 0.750 mL of Sedi-Tox diluent into negative control tubes and blank tubes





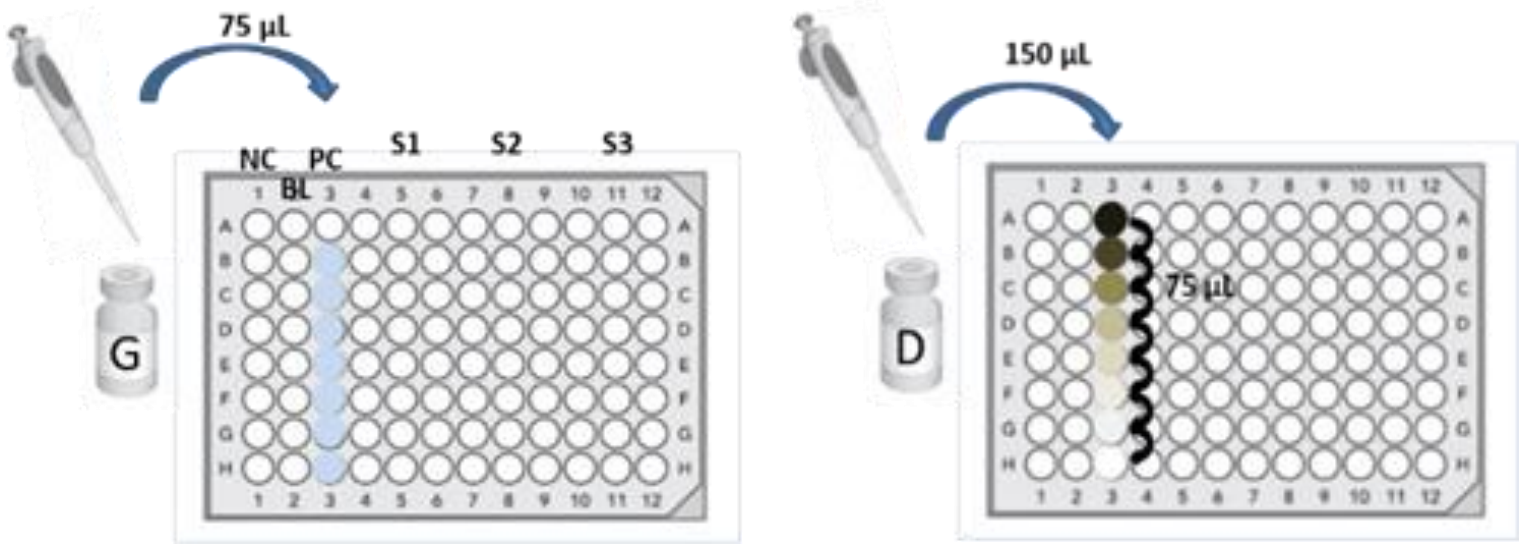
4. Dilute Sample

- Add homogenized sample suspension into the first tube in the dilution series
- Perform a set of 2-fold dilutions by transferring 0.750 mL of suspension from one tube to the next in the series
- Ensure that each solution is mixed well with the pipette prior to transferring any sample to the next tube.
- Remove 0.750 mL from the final tube in the series and discard



5. Plate Positive Control

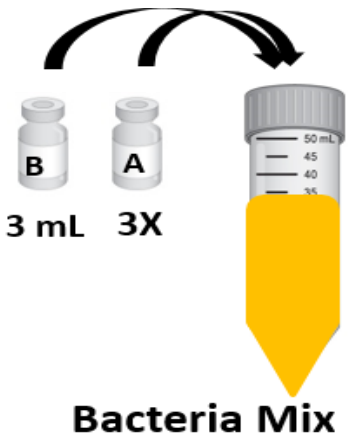
- Prepare result plate by preparing one column of a 2-fold dilution series of the positive control (vial D)
- This control will change depending on the bacterial strain used
- Use Sedi-Tox sample diluent and prepare the dilution series in the same way as the sample dilution series was prepared
- Discard 75 µL from the last well in the column



6. Reagent Preparation

- Prepare the bacterial reagent by adding 3 mL of the rehydrated stock culture to 3 bottles of the reaction mixture (vial A) in a sterile tube
- Mix this diluted reagent thoroughly. The bacteria is now ready to be dispensed
- Dispense 0.750 mL of the reagent into all sample and control tubes
- Dispense 75 µL of the reagent into all wells of the positive control column on the microplate

1.



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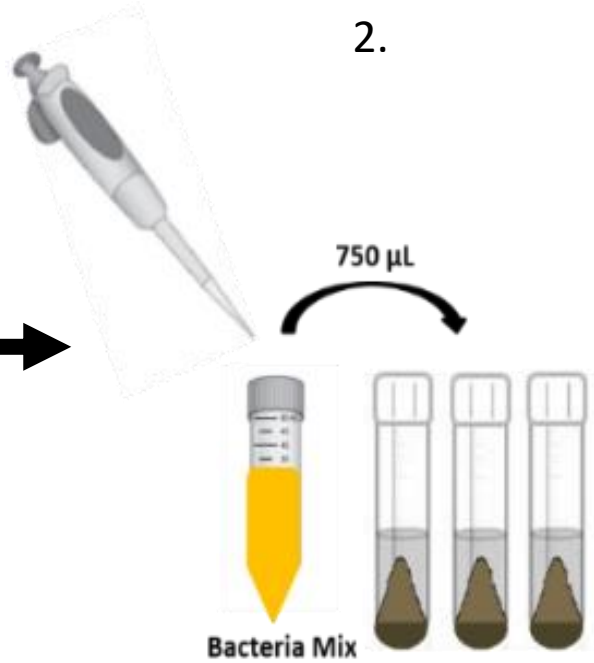

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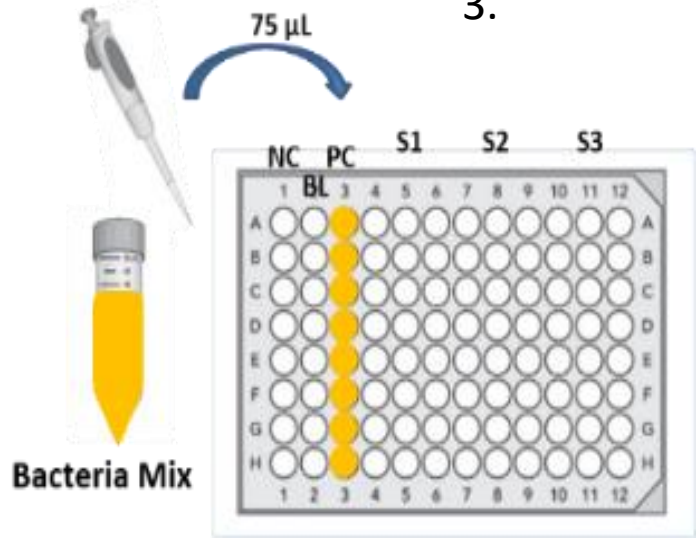
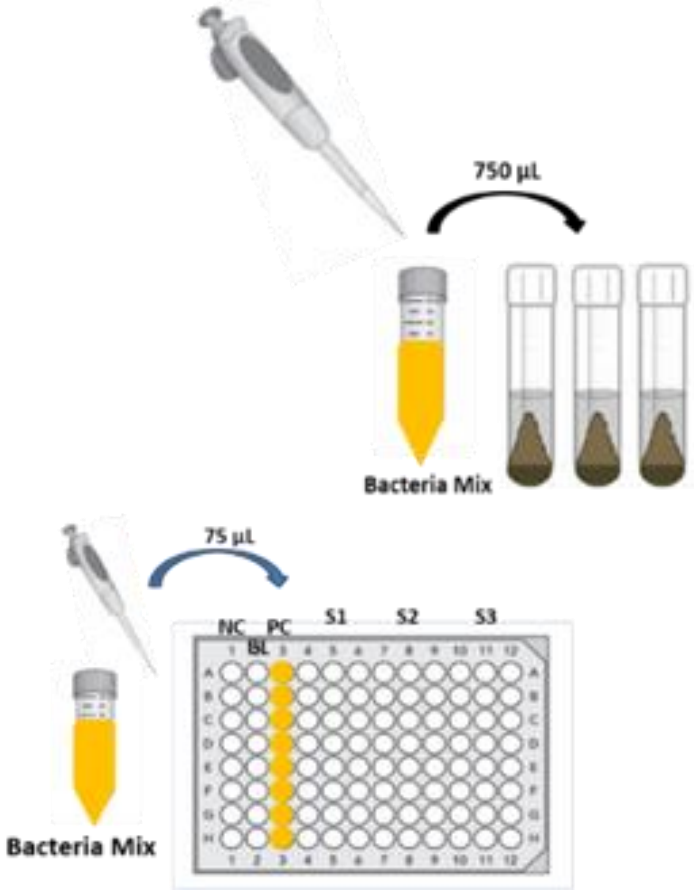
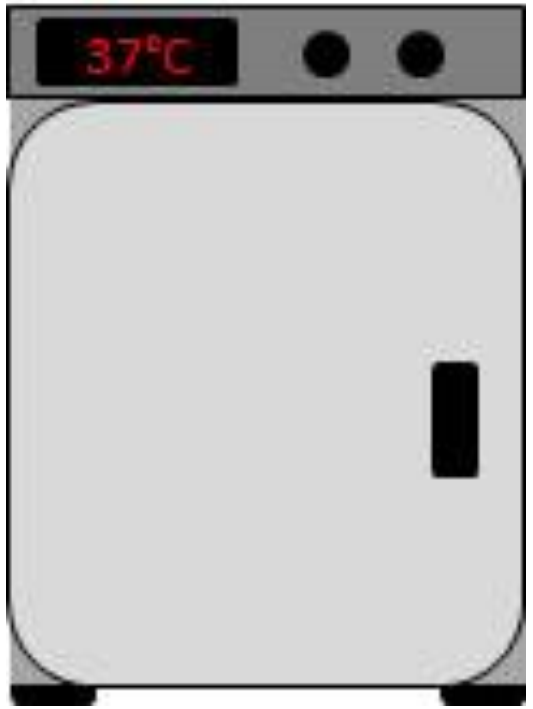


7. Incubation

- Incubate tubes and microplate at 37 °C for 2 hours with shaking or rotation
- If volatile components are suspected in the sample soils, parafilm the tops of the tubes to prevent evaporation
- After two hours remove the tubes and plate

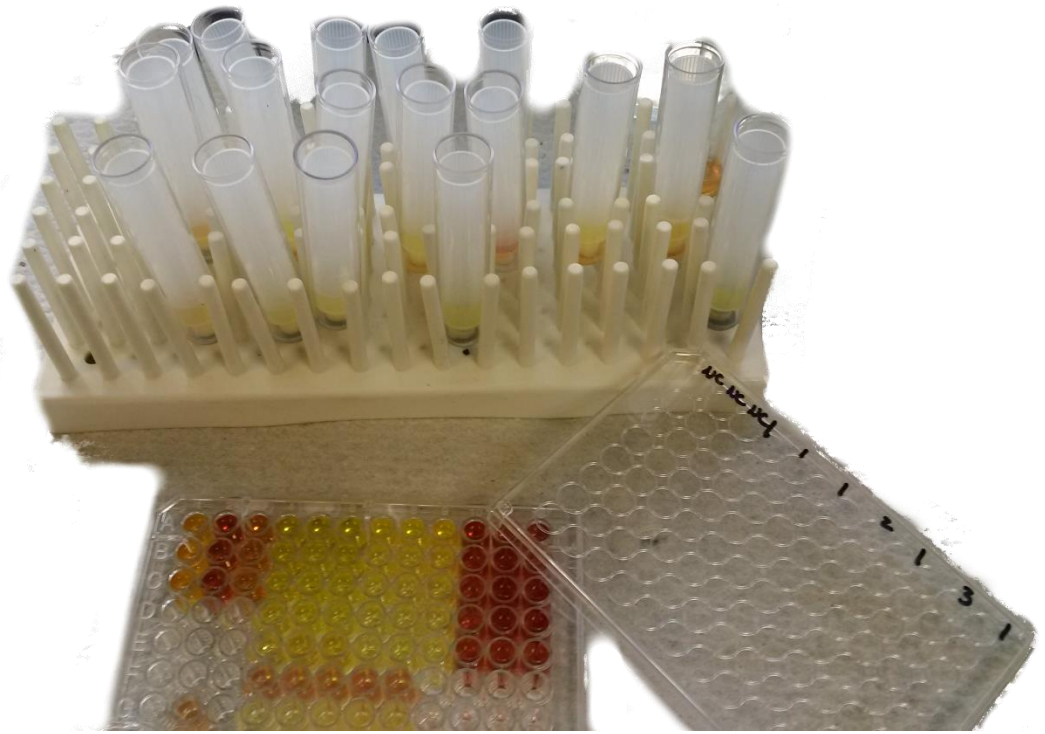




Incubate at 37°C
For 2 hours

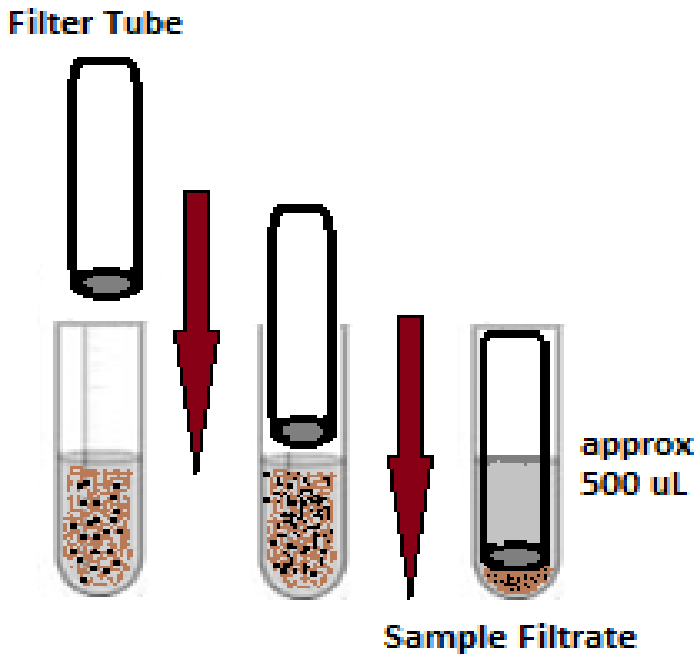
Analysis Options

- You have either purchased the qualitative or the quantitative method.
- There are two different procedures for analysis depending on your application
- After your incubation is complete follow the steps for
  - **Qualitative (QA Steps 8-11)**
  - **Quantitative (QN Step 8-13)**



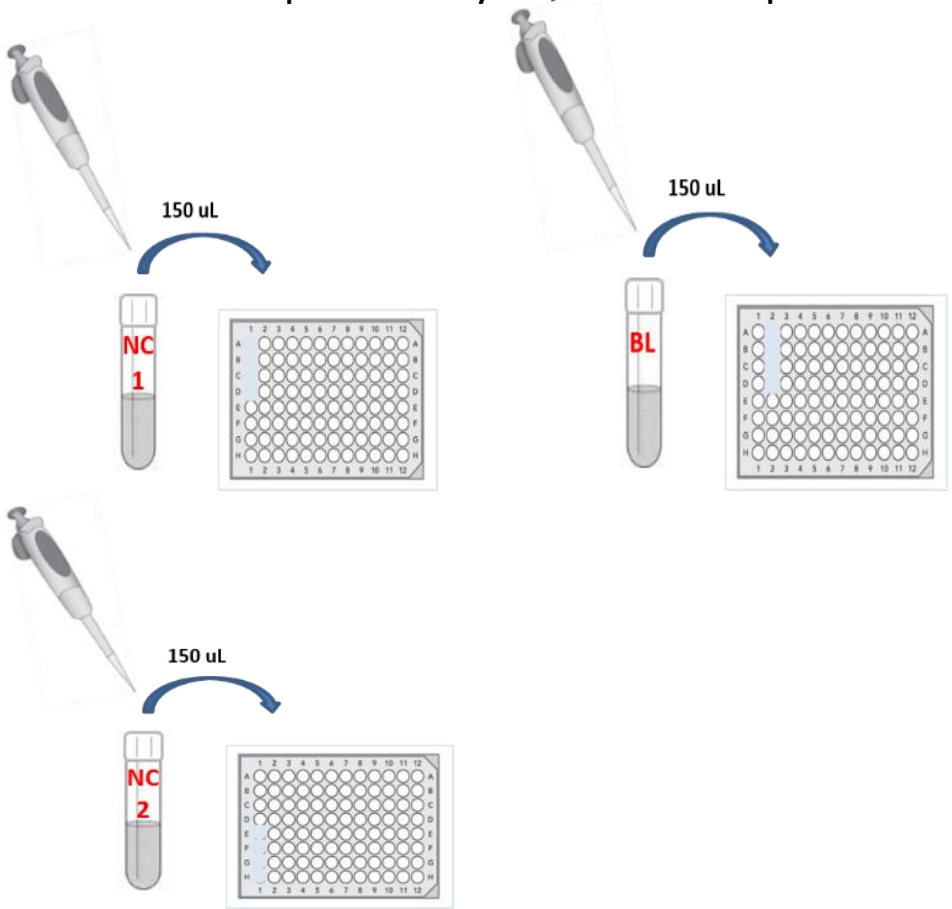
8. QA Filtration and Chromogen Addition

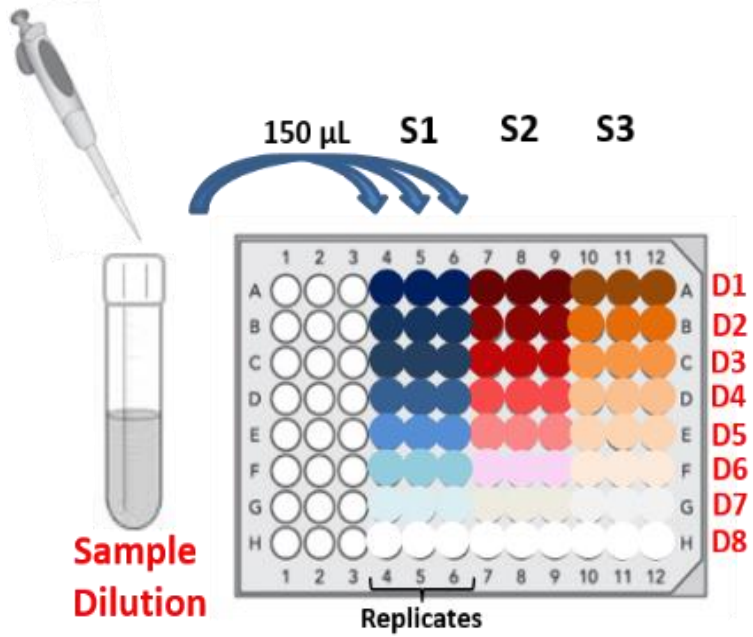
- After incubation is complete remove the tubes and plate
- Place disposable filters in the tops of the tubes
- Push down slowly and filter the larger particulate from the samples
- Some small particulate may get through the filter. This is normal
- Filter enough of the sample so that approximately 500 µL of filtrate remains



9. QA Filtration and Chromogen Addition

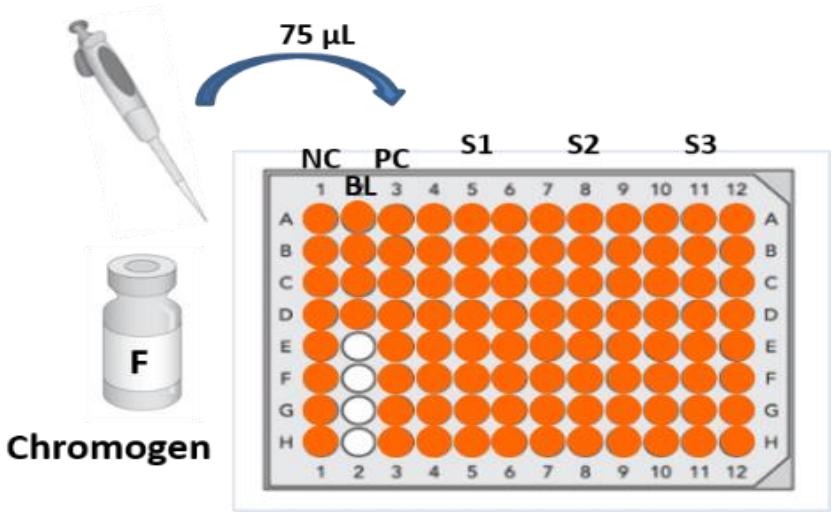
- Transfer 150 µL from each sample and control tube into the corresponding wells of the microplate.
- EBPI recommends plating triplicates for each dilution level to enhance the data collected.
- Transfer 150 µL of the blank tube into the corresponding wells of the microplate as shown below
- Use a fresh tip for every separate sample or control

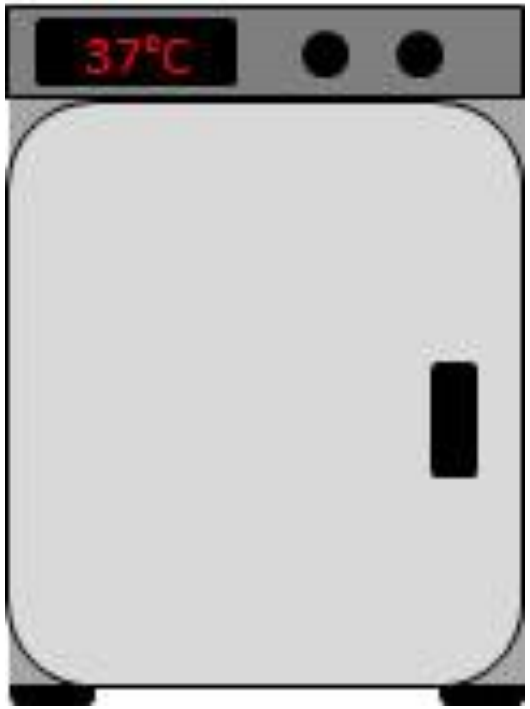




10. QA Chromogen Addition

- Remove the seal from the bottle of blue chromogen (vial F)
- Transfer 75 µL of chromogen to each well in the microplate that contains a sample or control
- Incubate the plate at 37 °C to increase the speed of the chromogenic reaction
- Look for a colour change before removing the plate





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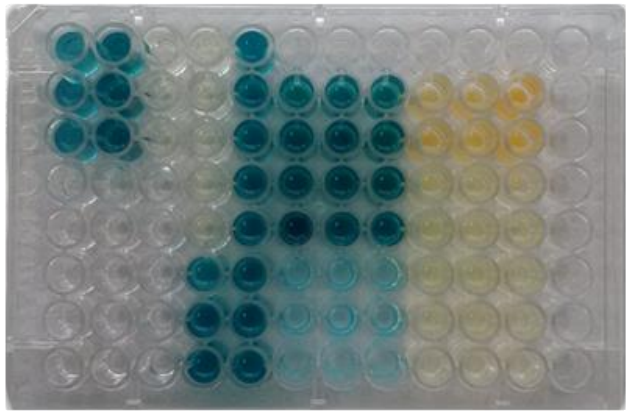
11. QA Result Interpretation

- Blue colour should develop in the wells that are non toxic. A deeper blue colour should be observed in the wells of the negative control.
- Record the qualitative observations that you see in **Table 1**. Comparisons for the amount of colour development in each sample dilution should be made with the negative control wells.
- The average colour development for each sample dilution be taken by observing all three replicates. Colour development between the three replicates should be very close and the replicates are used to eliminate erroneous outliers despite the analysis being qualitative in nature.

		Response
Sample	Dilution	(-,+,++,+++)
1	1	
	2	
	3	
	4	
	5	
	6	
	7	
	8	
2	1	
	2	
	3	
	4	
	5	
	6	
	7	
	8	
3	1	
	2	
	3	
	4	
	5	
	6	
	7	
	8	

	Toxicity
Sample #	SediTox EC_{100} (mg/mL)
1	
2	
3	



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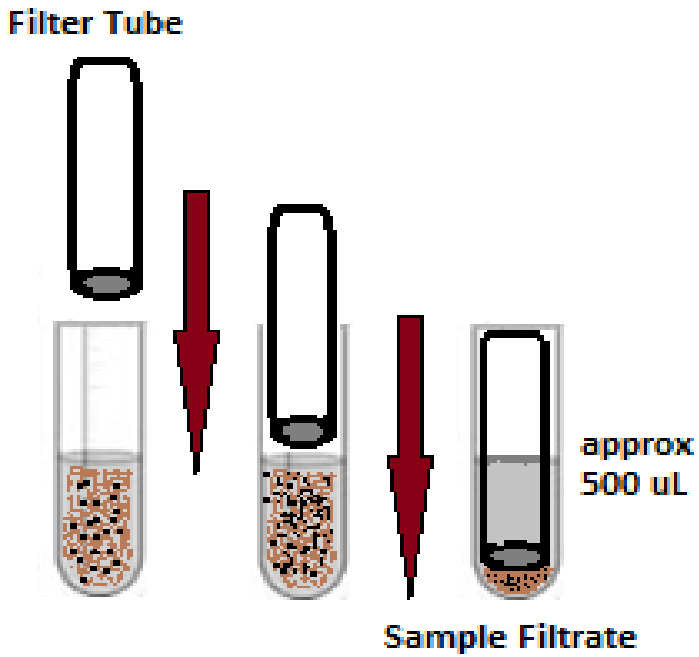
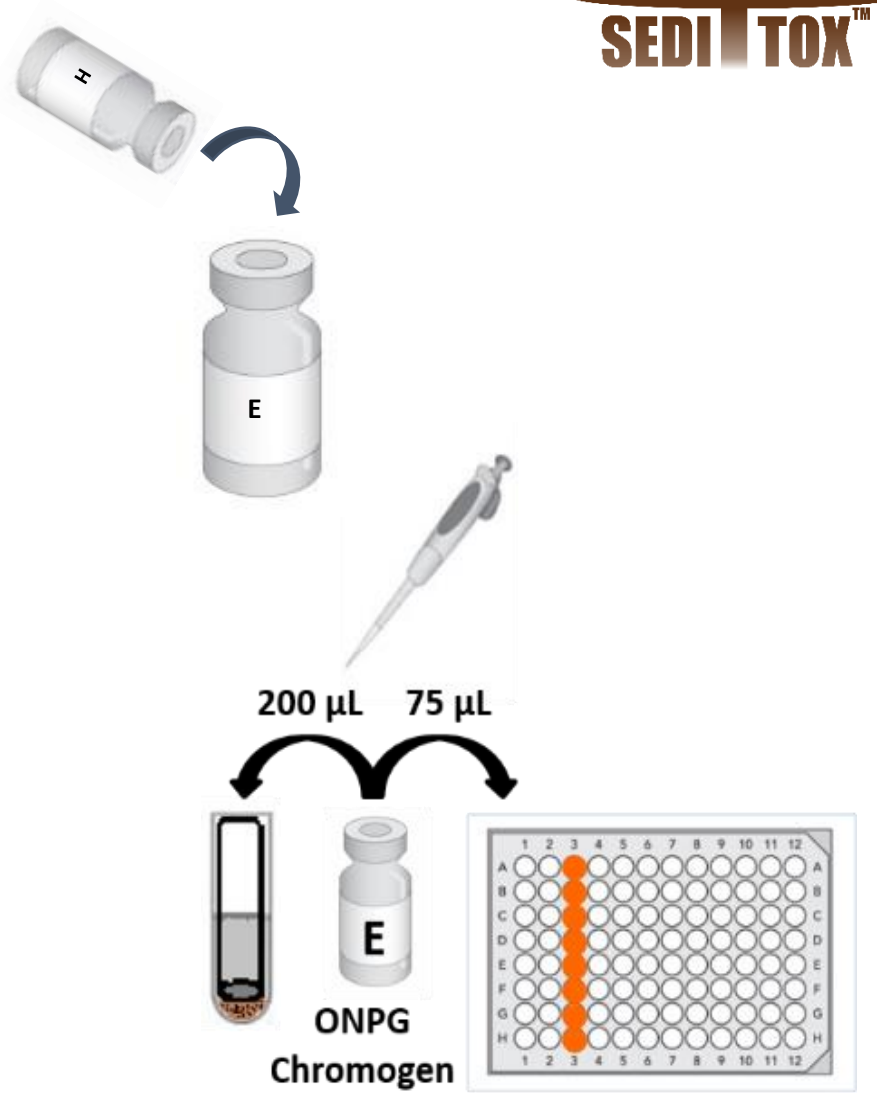
8. QN Filtration and Chromogen Addition

- After incubation is complete remove the tubes and plate
- Place disposable filters in the tops of the tubes
- Push down slowly and filter the larger particulate from the samples
- Some small particulate may get through the filter. This is normal
- Filter enough of the sample so that approximately 500 µL of filtrate remains



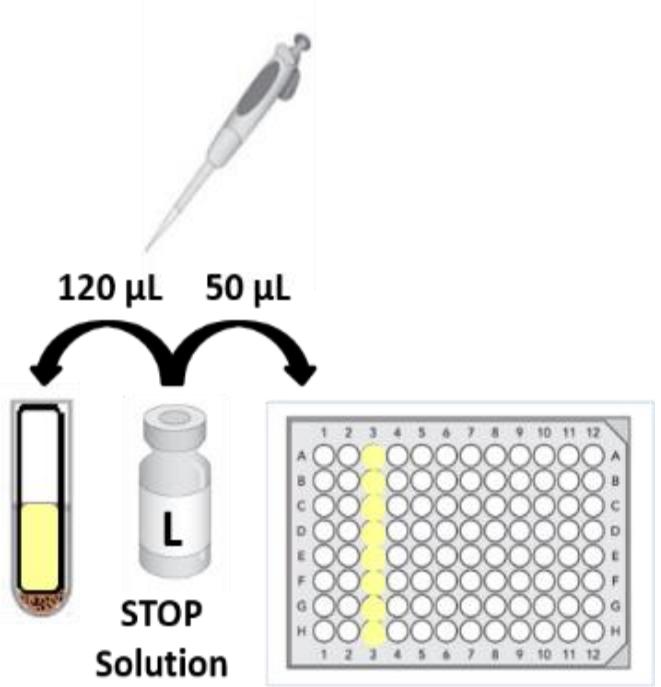
9. QN Filtration and Chromogen Addition

- Remove the seal from the bottle of ONPG chromogen (E). Pour the entire contents of Chromogen Diluent (H) into the bottle. Shake well to dissolve any particulate and leave the chromogen in the dark at room temperature until use.
- Pipette 200 µL of chromogen into each tube containing filtered negative controls, blanks and samples.
- Pipette 75 µL of chromogen into each well of the plate containing positive control samples (3A-3H).
- Incubate both the tubes and microplate at 37 °C for at least 1 h.
- Monitor the tubes and plate for colour development. You should start to see some yellow colour in the tubes and wells.



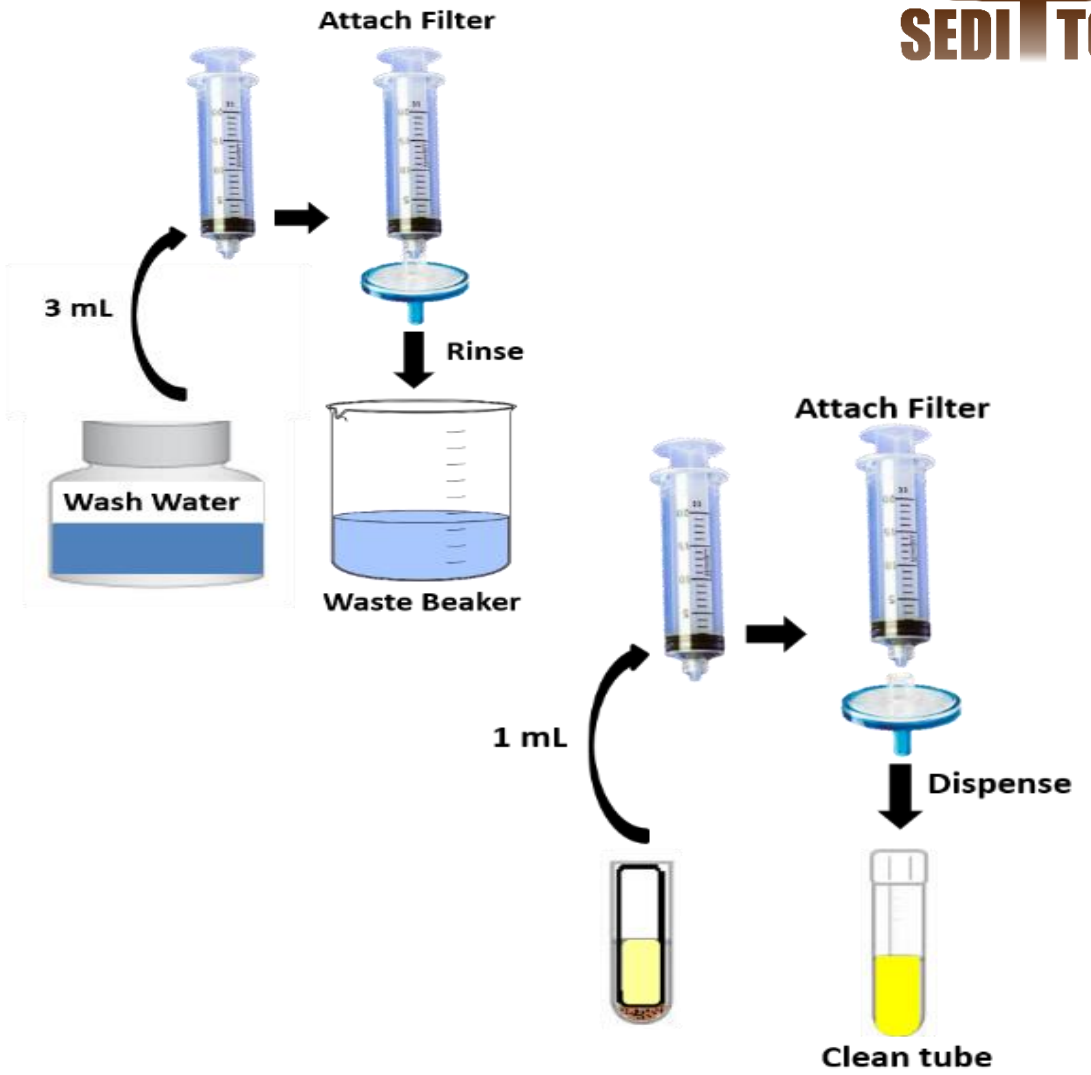
10. QN Filtration and Chromogen Addition

- If colour has developed, open the bottle of stop solution (L) and pipette 120 µL of stop solution (L) into each tube
- Pipette 50 µL of stop solution into each well of column 3 (3A-3H).
- Stop solution will sharpen the colour difference between samples and increase β-gal expression in the tubes.



11. QN Syringe filter assembly and initial wash

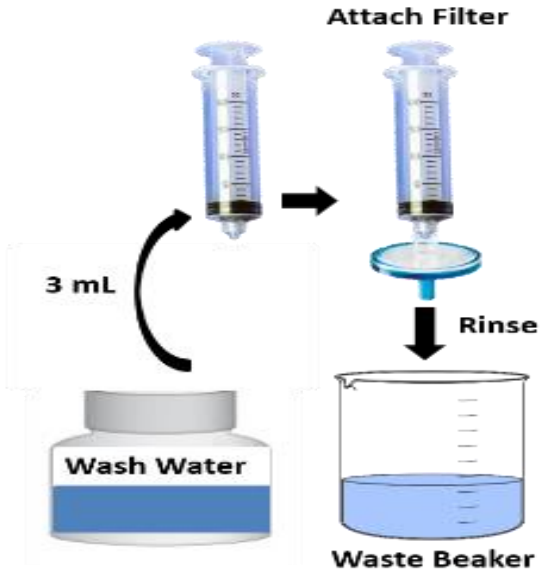
- Assemble the syringe filtration unit.
- Draw up 3 mL of wash water, remove the syringe tip, put on the filter and push the water through the filter.
- It is important that the same amount of water is used to wash the filter and syringe between samples.
- Beginning with the blank sample, attach the syringe tip, draw up approximately 1 mL of liquid into the syringe. Turn the syringe tip up towards the sky, remove the syringe and place the pre-rinsed syringe filter on the syringe.
- Expel the entire contents of the syringe through the filter into a clean, dry, labelled tube. Ensure that you have at least 600 µL of filtrate in the tube.

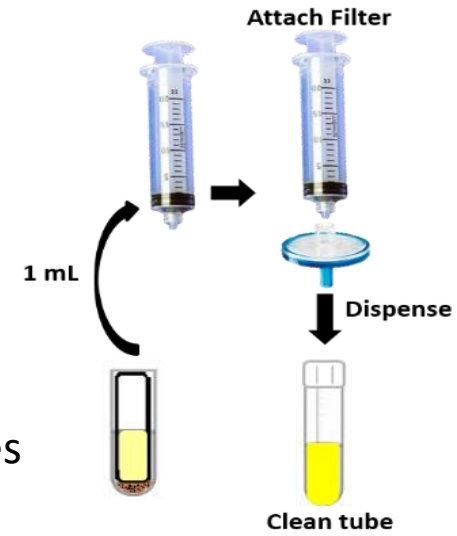


12. QN Transfer of Samples and Controls

- Remove the filter, draw up 3 mL of wash water into the syringe, replace the filter tip and rinse the filter into a waste beaker as before.
- Each rinse will wash through any remaining chromogen and should ensure that approximately the same amount of residual water is kept on the filter so that each sample filtrate is diluted by the same amount. This also means that a filter can be re-used for all dilutions of a given sample.
- Attach the syringe tip and repeat the transfer and wash procedure for each of the negative control tubes.

Reminder: The filter and syringe should be washed with the same amount of water in between each transfer.



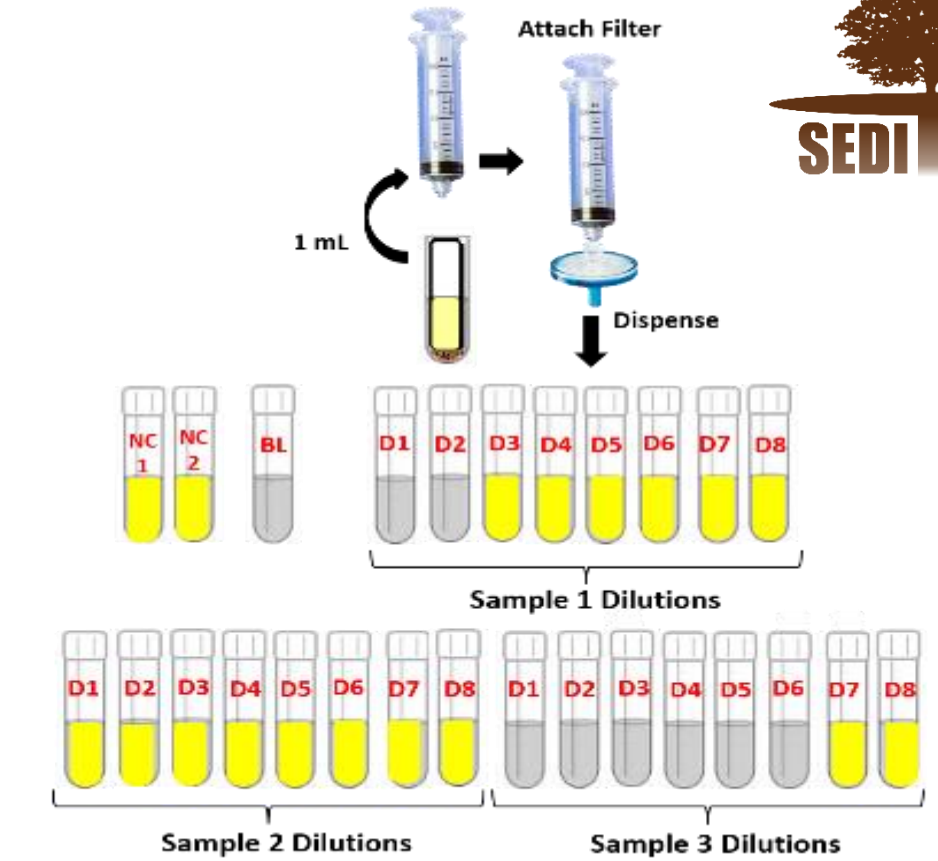


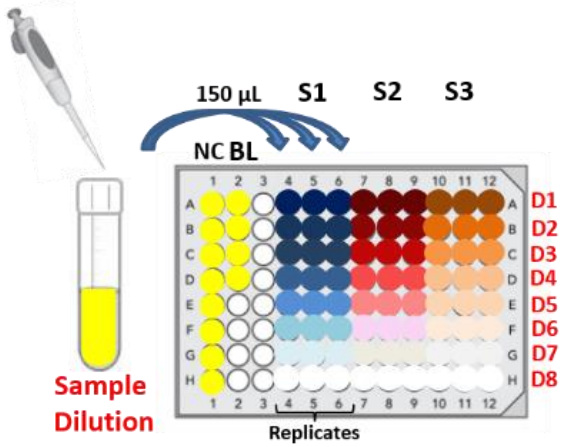
Control tubes

13. QN Transfer of Samples and Controls

- Using a new filter and syringe for each sample, repeat the transfer procedure for each sample dilution.
- Start with the most concentrated sample (least amount of colour development) and end with the most dilute sample.
- You have been provided with enough filters and syringes to use fresh components for each sample. Remember to wash each new filter with 3 mL of water prior to transferring the first sample dilution.
- After the transfers are complete, you will have a series of test tubes with clean samples are dilutions containing approximately 500 µL of volume in each.

- Pipette 150 µL of the filtrate from each tube into the corresponding wells of the provided microplate. For each sample dilution, three replicates should be dispensed as previously stated in the qualitative procedure.





14. QN Result Interpretation

- Once all the samples are dispensed, read the absorbance at 405 nm in a spectrophotometer
- Record the values in the table on the right or an excel spreadsheet.
- Calculate Toxicity factors **(TF)** for the unknown sediment or soil sample by:

1. Calculate the average value for the blank samples (background absorbance from media, diluent and chromogen)

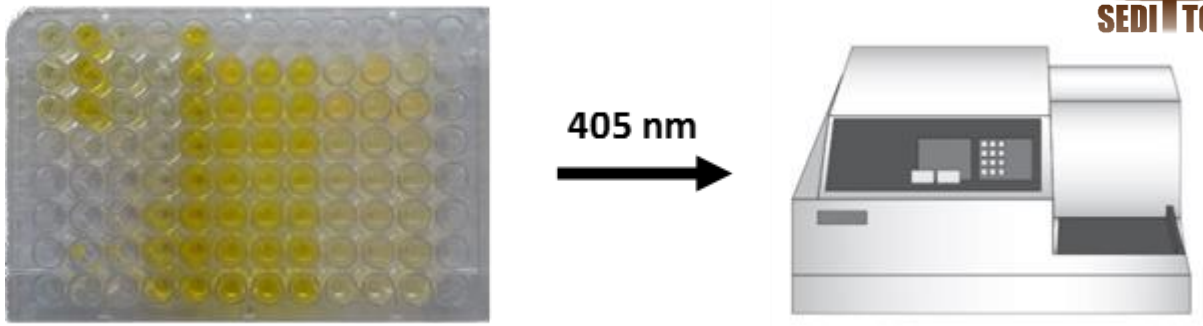
$$A_{405}BL_{avg} = (A_{405}BL1 + A_{405}BL2 + A_{405}BL3 + A_{405}BL3)/4$$

2. Calculate the average colour from the negative control samples

$$A_{405}NC_{avg} = (A_{405}NC + A_{405}NC + A_{405}NC + A_{405}NC + A_{405}NC + A_{405}NC + A_{405}NC + A_{405}NC)/8$$

3. Calculate the toxicity factor for each replicate of each sample dilution. The toxicity factor of **Sample 1 (S1)** is calculated using the following formula

$$TF = 100 - 100[(A_{405}S1 - A_{405}BL_{avg})/(A_{405}NC_{avg} - A_{405}BL_{avg})]$$



	Abs 400 nm						Abs 400 nm				
Sample	Dilution	Rep 1	Rep 2	Rep 3	Rep 4	Sample	Dilution	Rep 1	Rep 2	Rep 3	Rep 4
NC1	1					Sample 2	4				
NC2	1						5				
Blank	1						6				
Sample 1	1						7				
	2						8				
	3					Sample 3	1				
	4						2				
	5						3				
	6						4				
	7						5				
	8						6				
Sample 2	1						7				
	2						8				
	3										

When all TF values have been calculated, determine the average TF for each dilution level and associated standard deviation

The TF values for the sample dilutions can now be plotted with the positive control TFs to provide comparative dose response relationships and assess toxicity