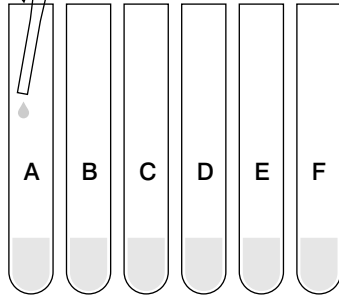
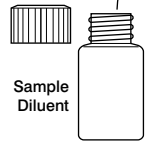


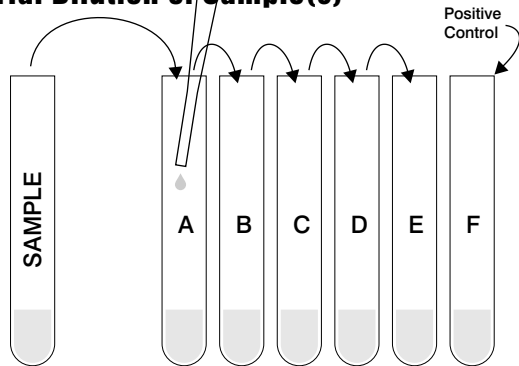
# AbraTox-Cam Acute Toxicity Procedure

## 1. Addition of Sample Diluent



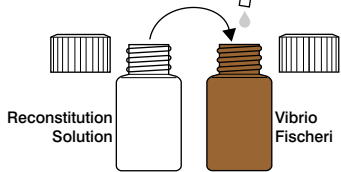
Dispense 2 mL of Sample Diluent (distilled water) into each dilution tube (A-F).

## 2. Serial Dilution of Sample(s)



Perform 5 sequential serial 1:1 dilutions of the sample to be tested by transferring 2 mL of sample into tube A, followed by mixing. Then transfer 2 mL of the contents of tube A into tube B. Serially dilute through tube E. Pipette 200  $\mu$ L of positive control into tube F.

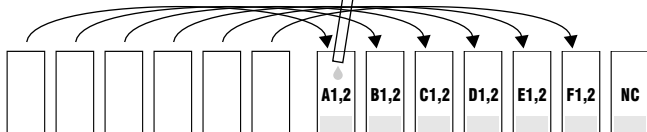
## 3. Hydration of Vibrio Fischeri



Add 2.5 mL of **cold** Reconstitution Solution into the Vibrio fischeri reagent vial, replace cap and mix by swirling for about 30 seconds. Incubate at 4°C for 30 minutes.



## 4. Addition of Samples to test Cuvettes

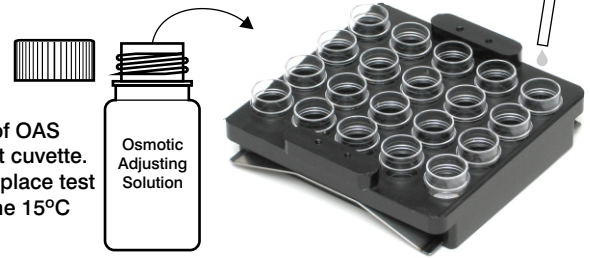


While Vibrio Fischeri reagent is incubating, perform the following:

- It is recommended that each test point be performed in duplicate, therefore, add 800  $\mu$ L of each sample dilution (A-E), positive control (F), negative control (NC) into duplicate cuvettes (A1, A2, B1, B2...F1, F2)
- Add, in duplicate, 800 $\mu$ L of Sample Diluent to Negative Control (NC) cuvettes.
- Place cuvettes into cassette.

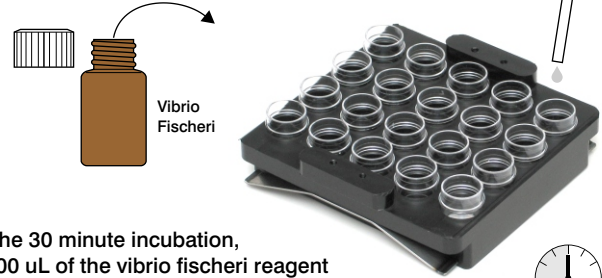


## 5. Addition of Osmotic Adjusting Solution



Add 100  $\mu$ L of OAS into each test cuvette. Mix well and place test cuvettes in the 15°C incubator.

## 6. Addition of Vibrio Fischeri reagent



After the 30 minute incubation, add 100  $\mu$ L of the vibrio fischeri reagent to all cuvettes. Mix well and place in the incubator to incubate 15-60 minutes.

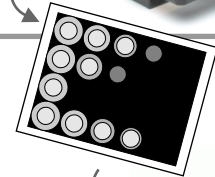
## 7. Load and Record

- After the 15-60 minute incubation, load the cassette into the AbraTox camera, pull out the dark slide and press the lever to the on position.
- Expose film for 10 minutes.
- Slide back the dark slide.
- Remove film from camera and allow to develop for 3-5 minutes then pull away film from backing. Wipe away any remaining film developing gel from the film perimeter using a paper towel being careful not to disturb the photograph.



## 8. Measure and Calculate

- Scan photo using a scanner.
- Measure light intensity for each sample control and sample dilution using the software provided.
- Calculate % inhibition for each sample as compared to negative control and calculate EC50 using the software provided.



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