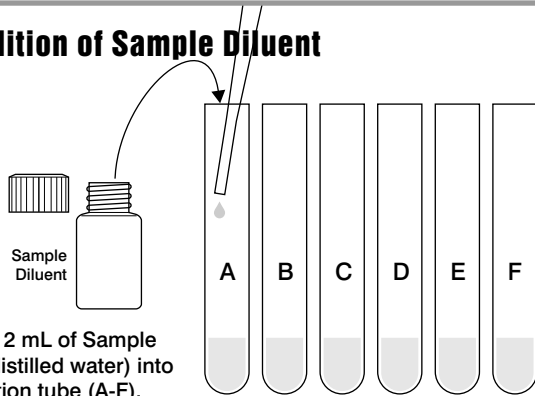


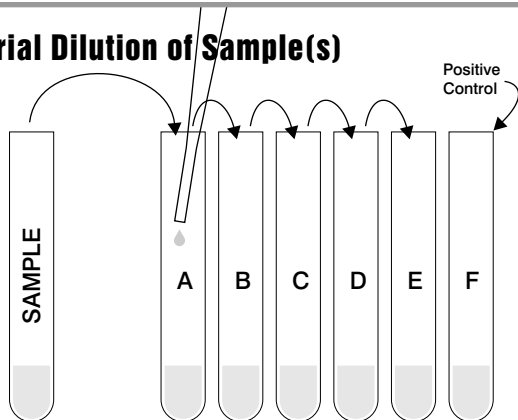
# AbraTox Acute Toxicity Procedure

## 1. Addition of Sample Diluent



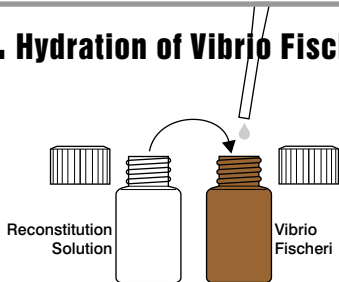
Dispense 2 mL of Sample Diluent (distilled water) into each dilution tube (A-F).

## 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 uL of positive control into tube F.

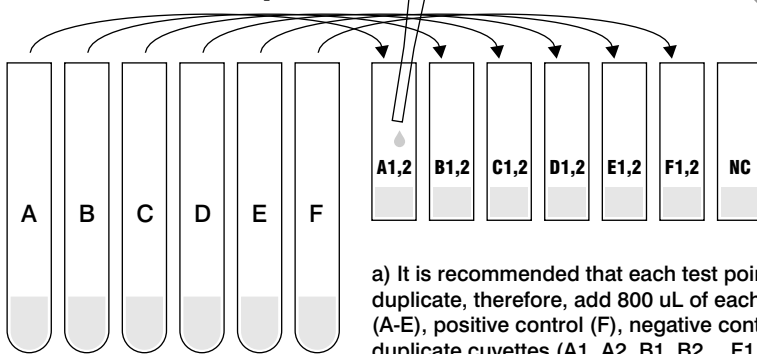
## 3. Hydration of Vibrio Fischeri



Add 2.5 mL of 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.



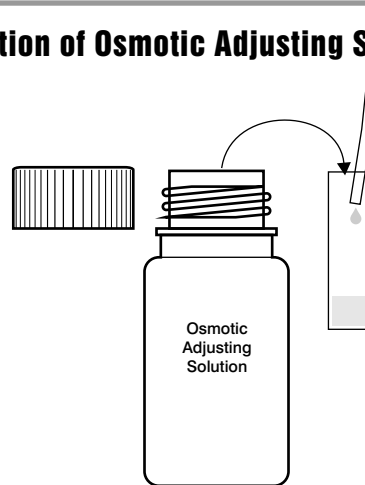
## 4. Addition of Samples to test Cuvettes



While Vibrio Fischeri reagent is incubating, perform the following:

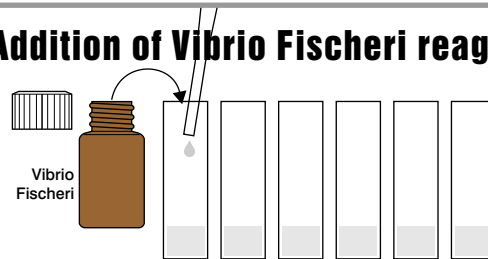
- It is recommended that each test point be performed in duplicate, therefore, add 800 uL of each sample dilution (A-E), positive control (F), negative control (NC) into duplicate cuvettes (A1, A2, B1, B2...F1, F2)
- Add, in duplicate, 800uL of Sample Diluent to Negative Control (NC) cuvettes.

## 5. Addition of Osmotic Adjusting Solution



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

## 6. Addition of Vibrio Fischeri reagent

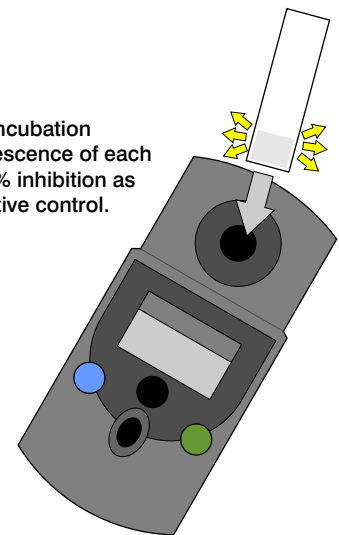


After the 30 minute incubation, add 100 uL of the vibrio fischeri reagent to all cuvettes. Mix well and place in the incubator to incubate 15-60 minutes.



## 7. Measure

After 15 to 60 minute incubation measure the bioluminescence of each cuvette and calculate % inhibition as compared to the negative control.



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