

FLUOROMETPAD™ INSTRUCTIONS

The FluoroMetPAD™ kit is specific for heavy metal toxicity and is based on enzyme inhibition in a bacterial strain by bioavailable heavy metals in aqueous samples. Bacterial response to a toxic sample is conveniently observed on the test pad. The intensity of the fluorescence on the pad is inversely proportional to the sample toxicity.

One MetPAD™ test kit contains one vial of freeze-dried **BACTERIAL REAGENT**, one vial of **DILUENT**, one vial of **BUFFER**, one vial of **POSITIVE CONTROL**, and three **ASSAY PADS**. The equipment required to perform the MetPAD™ includes: test tubes with caps, pipettors with tips, an incubator and a long-range UV lamp (Black light). Additional equipment is needed for solids testing.

CAUTION: AS WITH ALL MICROBIOLOGICAL AND TOXIC SPECIMENS, SAFETY PROCEDURES SHOULD BE FOLLOWED. USE GLOVES TO HANDLE KIT AND SAMPLES. AUTOCLAVE BOTTLES AND PIPET TIPS BEFORE SAFE DISPOSAL. PLEASE NO MOUTH PIPPETING.

The FluoroMetPAD™ toxicity assay procedure is completed as follows:

Preparation of Solid Samples (e.g. soils, sediments, sludges)

Use the following extraction technique for solid (e.g., soil, sediment) samples.

- I. Suspend soil in MilliQ water at a ratio of 1:2.5 (soil/water)
- II. Shake solids mixture at 200 strokes per minute for 2 hours at room temperature.
- III. Centrifuge solids mixture at 3000 rpm for 30 minutes.
- IV. Use the supernatant or water elutriate for **FluoroMetPAD™ Toxicity Assay**

Preparation of Aquatic samples

- Step 1 First, test the pH of each sample. If the pH is outside the range of 5.0-8.0, adjust the pH to pH=6.5 with NaOH (1N) or HCL (1N). Record the dilution made.

- Step 2 Use undiluted sample for toxicity testing, or prepare dilutions of each test sample as desired using the **DILUENT** for EC₅₀ calculations. Mark each tube with the sample number and dilution factor.

Preparation of Bacterial Reagent

NOTE: The **RECONSTITUTED BACTERIAL REAGENT** will last for approximately 2 days (stored in a refrigerator).

- Step 3 Add 5.0 ml of **DILUENT** to the vial containing the **BACTERIAL REAGENT**.
- Step 4 The **BACTERIAL REAGENT** is hand shaken or vortexed for approximately 30 seconds to obtain a uniform suspension.. Incubate the **RECONSTITUTED BACTERIAL REAGENT** at room temperature (18°C -25°C) for 15 minutes prior to performing the toxicity assay.

FluoroMetPAD™ Toxicity Assay

Each FluoroMetPAD™ test kit can measure the toxicity of approximately 8 undiluted samples in duplicate, including a negative and positive control, also in duplicate. Each FluoroMetPAD™ test kit is sufficient to run one complete toxicity tests (i.e., EC₅₀). A complete toxicity test includes the undiluted sample in duplicate, 4 sample dilutions in duplicate, and controls in duplicate (positive and negative).

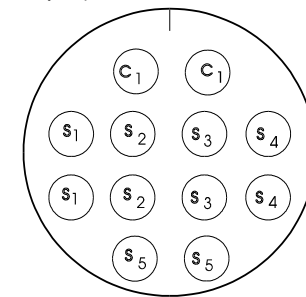
- Step 5 Add 0.9 ml of each test sample or test sample dilution to clean glass tubes and label with sample number and dilution factor.
- Step 6 Add 0.1 ml of **RECONSTITUTED BACTERIAL REAGENT** to the tubes containing 0.9 ml of the test sample or test sample dilution. Vortex each test sample for 30 seconds.
- Step 7 To prepare the negative controls, add 0.1 ml of **RECONSTITUTED BACTERIAL REAGENT** and 0.9 ml of **DILUENT** to a clean glass tube. Label tube as control. Vortex tubes for 30 seconds.

CAUTION: The positive control contains copper sulfate (1 mg/L as Cu²⁺) and is toxic. Handle with care.

- Step 8 To prepare the positive controls, add 0.1 ml of **RECONSTITUTED BACTERIAL REAGENT** to 0.9 ml of

POSITIVE CONTROL to clean glass tubes. Label tubes as positive control. Vortex tube for 10 seconds.

- Step 9 Incubate all tubes at 35°C for 90 min using an incubator.
- Step 10 At the end of the incubation period add 0.1 ml of **BUFFER** to each test sample, test sample dilution, and the controls. Vortex tubes thoroughly.
- Step 11 Pipette 10 µL from each test sample, test sample dilution, and the controls onto the **ASSAY PAD** in duplicate You may remove the **ASSAY PAD** from the petri dish for easier pipetting. Place the negative control duplicates at the top of the pad and mark with a pen for later identification. Place drops of the **ASSAY PAD** as shown in the figure below. Test samples should be spaced as evenly as possible.



C - Control

S - Sample

- Step 12 Place the **ASSAY PAD** inside the petri dish and incubate at 35°C until fluorescence develops in the negative controls (approx. 30 - 90 min).
- Step 13 After the incubation period, using a long-range UV lamp (black light), observe the **intensity of the fluorescent spots**, which indicates the level of enzyme activity. The sample will remain non fluorescent in the positive control or in the presence of a very toxic sample. The sample will turn fluorescent in the negative control or in the non-toxic samples.

CAUTION: Properly and safely dispose of all remaining reagents.