

MetPAD™ INSTRUCTIONS

The MetPAD™ 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 purple color 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 four **ASSAY PADS**. The equipment required to perform the MetPAD™ includes: test tubes with caps, pipettors with tips, and a regular lab incubator. 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 MetPAD™ 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 about 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 **MetPAD™ 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 around pH=6 with NaOH (1N) or HCL (1N). Record the dilution made.
- Step 2 Use undiluted samples for toxicity testing (qualitative test). To estimate LOEL (Lowest Observed Effect Level), prepare dilutions of each test sample as desired using the **DILUENT**. 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 (store in a refrigerator).
- Step 3 Add 3.0 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.

MetPAD™ Toxicity Assay

Each MetPAD™ test kit can measure the toxicity of approximately 12-13 undiluted samples in duplicate, including a negative and positive control, also in duplicate. Each MetPAD™ test kit is sufficient to run one complete toxicity tests (i.e., LOEL estimation). A complete toxicity test includes the undiluted sample in duplicate, 4 sample dilutions in duplicate, and controls in duplicate (positive and negative).

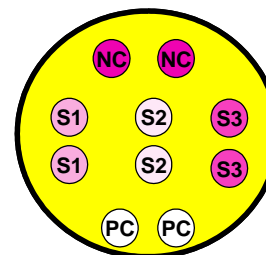
- Step 5 Add .9 ml of each test sample or test sample dilution to clean labeled glass tubes.
- Step 6 Add 0.1 ml of **RECONSTITUTED BACTERIAL REAGENT** to the tubes containing 0.9 ml of the test sample or test sample dilution. Vortex each test sample for 30 seconds.
- Step 7 To prepare the negative controls, add 0.1 ml of **RECONSTITUTED BACTERIAL REAGENT** and 0.9 ml of **DILUENT** to a clean glass tube. Label tube as negative control. Vortex tubes for 10 seconds.

CAUTION: The positive control contains copper sulfate (1 mg/L as Cu²⁺) and should be toxic.

- Step 8 To prepare the positive controls, add 0.1 ml of **RECONSTITUTED BACTERIAL REAGENT** to 0.9 ml of **POSITIVE CONTROL** in 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 dilutions, and the controls. Vortex tubes thoroughly.

- Step 11 Pipette 10 µL from each test sample, test sample dilution, and the controls onto the **ASSAY PADS** in duplicate You may remove the **ASSAY PADS** from the petri dishes 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.



NC = Negative Control

PC = Positive Control

S = Sample

- Step 12 Place the **ASSAY PAD** inside the petri dish and incubate at 35°C until color develops in the negative controls (approx. 30 - 90 min).

- Step 13 After the incubation period, observe the intensity of the red/purple color, which indicates the level of enzyme activity. The sample will remain yellow in the positive control or in the presence of a very toxic sample. The sample will turn red/purple in the negative control or in the non-toxic samples.

MetPAD Scale: Non toxic: 5+; 100% toxic: 0+

CAUTION: Properly dispose of all remaining reagents.