

MetSTICK™ INSTRUCTIONS

The MetSTICK™ kit is specific for heavy metal toxicity.

In the MetSTICK heavy metal toxicity test we use **immobilized test bacteria** to determine the heavy metal toxicity of solids such as sediments, soils and, possibly, biosolids. As for MetPAD and MetPLATE the test is specifically sensitive to heavy metals.

The test bacteria (also used in MetPAD/MetPLATE) are solidly immobilized on a plastic slide.

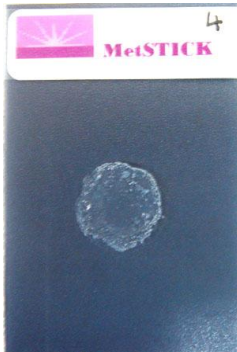


FIGURE 1

Plastic slide with immobilized bacteria

The slides are attached to a wooden stick which is inserted in the sediment to the desired depth and then allowed to remain in the sediment for a period of 4 hours. One can attach several slides to the wooden stick to determine the heavy metal toxicity in both water and sediment simultaneously. After the 4-hr time period, the wooden stick is removed from the sediment and brought back to a laboratory for enzyme assay and determination of percent inhibition.

MetSTICK Kit: One MetSTICK™ test kit contains **12 PLASTIC SLIDES WITH IMMOBILIZED BACTERIA** on the labeled side of each slide, one vial of freeze-dried and buffered **FLUOROGENIC SUBSTRATE**, one bottle of moderately hard water. The equipment required to perform the MetSTICK includes: pipettors with tips, an incubator (35C), and a fluorometer

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

Preparation of Sediment samples

No preparation is necessary since the sediment is already saturated and tested mostly under *in situ* conditions

Preparation of Soil samples

Before using MetSTICK, the soil must be saturated with deionized water and allowed to reach equilibrium for a period of 4 hrs.

Preparation of Fluorogenic Enzyme Substrate

CAUTION: This solution is light sensitive and should be kept away from direct light (to avoid exposure to light, the substrate is supplied in a brown bottle).

Add 8 ml of deionized water to the **FLUOROGENIC SUBSTRATE** vial.

The **FLUOROGENIC SUBSTRATE** is then hand-shaken or vortexed for approximately 10 seconds, and allowed to stand at room temperature for a period of 15 min..

MetSTICK™ Toxicity Assay

Each MetSTICK™ test kit can be used to measure the toxicity of 4 samples in triplicate

- Step 1 **Original fluorescence of a slide:** To each slide, add 0.3 ml of the **RECONSTITUTED ENZYME SUBSTRATE** (gently dispense the substrate on top of the circle containing immobilized bacteria (see Figure 1).
- Step 2 Incubate the slides for 30 min at 35C.
- Step 3 After 30 min, gently mix thoroughly the substrate on top of the slide (**Caution: avoid touching the immobilized bacteria with the pipet tip**).
- Step 4 The fluorescence is measured, using a fluorometer microplate reader (360 nm excitation filter and 485 nm emission filter). Other fluorometers can be used to measure fluorescence. This is the fluorescence reading **BEFORE** exposing the slides to the toxic sediment or soil (**READING 1**).

- Step 5 Each slide is then washed with deionized water (the immobilized bacteria will not be washed out)
- Step 6 Each slide is inserted into a saturated soil or sediment to a desired depth *in situ*. The slide is left in the soil or sediment for a period of 4 hrs. The slide is retrieved from the toxic sample and gently washed with deionized water.
- Step 7 Then each slide is assayed for enzyme activity (Follow steps 1 to 4). The fluorescence reading obtained is **READING 2**
- Step 8 **Calculation of the % inhibition:**

The percent inhibition is calculated as follows:

$$(1 - \text{Reading 2}/\text{Reading 1}) \times 100$$

PLEASE NOTE

Suggestion concerning sediment toxicity:

To test sediment toxicity, a slide can be covered with a plexiglass screen found in home building stores. The covered slide is then attached to a wooden stick with a plastic tape to expose the immobilized bacteria *in situ* to the sediment. The wooden stick is inserted into the sediment to the desired depth and allowed to remain in the sediment for 4 hrs. Several slides could be attached to the wooden stick to determine metal toxicity in both the sediment and the water column, Following the 4-hr exposure period, the slides are assayed for enzyme activity in the laboratory. The percent inhibition is calculated according to the above Instructions.