

FluoroMetPLATE™ INSTRUCTIONS

The FluoroMetPLATE™ kit is specific for heavy metal toxicity. The FluoroMetPLATE™ kit 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 96-well microplate. The fluorescence intensity is inversely proportional to the sample toxicity. Fluorescence is measured using a fluorometer- microplate reader.

One FluoroMetPLATE™ test kit contains one vial of freeze-dried **BACTERIAL REAGENT**, one vial of freeze-dried **FLUOROGENIC SUBSTRATE**, 2 vials of **DILUENT**, one vial of **BUFFER**, one vial of **POSITIVE CONTROL**, and one 96 well **MICROPLATE**. The equipment required to perform the FluoroMetPLATE™ includes: test tubes with caps, pipettors with tips, an incubator, and a fluorometer.

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 FluoroMetPLATE™ toxicity assay procedure is completed as follows:

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

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 **FluoroMetPLATE™**

Toxicity Assay

Preparation of Aquatic samples

- Step 1 Test the pH of each sample. If the pH is outside the range of 5.0-8.0, adjust the pH to pH=6.0 with NaOH or HCl. 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 Fluorogenic Substrate

CAUTION: This solution is light sensitive and should be kept away from direct light

- Step 3 Add 10 ml of **BUFFER** to the **FLUOROGENIC SUBSTRATE** vial.
- Step 4 The **FLUOROGENIC SUBSTRATE** is then hand shaken or vortexed for approximately 10 seconds.

Preparation of Bacterial Reagent

- Step 5 Add **5 ml of DILUENT** to the vial containing the **BACTERIAL REAGENT**
- Step 6 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.

FluoroMetPLATE™ Toxicity Assay

Each FluoroMetPLATE™ test kit can measure the toxicity of approximately >25 undiluted samples in triplicate, including a negative, positive, and blank control, also in triplicate. Each test kit is sufficient to run complete toxicity tests (i.e., EC₅₀) for 4 samples. A complete toxicity test includes the undiluted sample in triplicate, 4--5 sample dilutions in triplicate, and controls in triplicate (positive, negative, and blank).

Step 7 Add 20µL of **RECONSTITUTED BACTERIAL REAGENT** to tubes containing 0.5 ml of the test sample or test sample dilution. Cap each test sample and vortex for 10 seconds.

Step 8 To prepare the negative controls, add 10 µL of **RECONSTITUTED BACTERIAL REAGENT** to 0.5 ml of **DILUENT** in clean glass tubes. Label tubes as negative control. Cap and vortex for 10 seconds.

CAUTION: The positive control contains copper sulfate and is toxic. Handle with care.

Step 9 To prepare the positive controls, add 10 µL of **RECONSTITUTED BACTERIAL REAGENT** to 0.5 ml of **POSITIVE CONTROL** in clean glass tubes. Label tubes as positive control. Cap and vortex for 10 seconds.

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

Step 10 To prepare the sample blanks, add 10 µL of **DILUENT** to 0.5 ml of the sample in clean glass tubes. Label tubes as blanks. Cap and vortex for 10 seconds.

Step 11 Incubate all the tubes in an incubator at 35°C for 90 minutes.

Step 12 After incubation, pipette 0.2 ml aliquots from each test tube onto the **MICROPLATE**, and add 0.1 ml of the **RECONSTITUTED FLUOROGENIC SUBSTRATE** to each test well.

Step 13 Incubate the **MICROPLATE** at 35°C until fluorescence develops (1 - 3 hours) in the negative control .

Step 14 After the incubation period, observe the intensity of the fluorescence, which indicates the level of enzyme activity. The sample will remain non or weakly fluorescent in the positive control and in the presence of a very toxic sample. The sample will turn fluorescent in the negative control and in the non-toxic samples. Fluorescence intensity can be determined, using a fluorometer at an excitation wavelength of 360nm and an emission wavelength of 485nm.

Calculate the percent inhibition or EC₅₀ according to standard procedures.

CAUTION: Properly and safely dispose of all remaining reagents.