1. INTRODUCTION

EBPI Sediment Toxi-ChromoPad™ is a rapid bacterial-based colorimetric bioassay kit for the determination of toxicity of sediments, suspended sediments, soils and solid wastes. It is sensitive to a wide spectrum of toxic substances such as heavy metals, and organic and inorganic pollutants, and may be used to detect the presence of toxicants directly without solvent extraction. The assay is based on the ability of substances (toxicants) to inhibit the *de novo* synthesis of an inducible enzyme - β-galactosidase - in a highly permeable mutant of *E. coli*.

The sensitivity of the test is enhanced by exposing the bacteria to stressing conditions, after which they are rehydrated in a cocktail containing a specific inducer of β-galactosidase, and essential factors required for the recovery of the bacteria from their stressed condition. The activity of the induced enzyme is detected by the hydrolysis of a chromogenic substrate. Toxic materials interfere with the recovery process and thus with the synthesis of the enzyme and the colour reaction.

WARRANTY

The Sediment Toxi-ChromoPad™ kit or its components will be replaced if defective in manufacturing or packaging. Complete results of the control reactions should accompany all replacement claims.

2. HANDLING THE SEDIMENT CHROMOTEST KIT

Handle the Sediment Toxi-ChromoPad™ Kit and your tested samples as you would any potentially hazardous material.

Although the bacterial strain is not a known pathogen, it is advisable to sterilize the remains of the Sediment Toxi-ChromoPad™ Kit before disposal (use the included biohazard bag).

Due to the short incubation time and chemical configuration of the kit, sterile handling is not imperative.

The Sediment Toxi-ChromoPad™ Kit should be stored under refrigeration (2 to 8°C), and should be protected from high temperatures and temperature changes.

3. LIST OF TOXI-CHROMO-PAD COMPONENTS

Each Sediment Toxi-ChromoPad™ package contains bacteria, solutions need and all plastics required for assays.

The contents of the bottles in each kit are:

A) Reaction mixture - a cocktail containing an enzymatic inducer and co-factors required for the recovery of the bacteria from their stressed condition. (1 vial)

B) The Sediment Toxi-ChromoPad™ lyophilized bacteria - a highly permeable selected mutant of *E. coli*. (1 vial)

C) Rehydration solution - a solution to hydrate the bacteria. (1 vial)

D) Chromogenic pads - contain blue chromogenic substrate. (4 pads)

E) Disposable plastic pipets. (14 pipets)

F) Disposable test tubes. (24 tubes)

G) Plastic bag for incubation. (1 bag)

4. USING ONE SEDIMENT CHROMOTEST KIT

Each Sediment Toxi-ChromoPad™ package contains all the material necessary to test four samples, in five two-fold dilutions each, for semi-quantitative analysis. For qualitative screening, you may test up to 20 samples (with just one dilution) with the existing reagents (but additional pipets may be required).

5. SEDIMENT TOXI-CHROMOPAD™ PROCEDURE

This chapter describes the steps of the Sediment Toxi-ChromoPad™ procedure. Figures 1 and 2 illustrate the procedure. Be sure to include a control with each test.

Before you start, set up series of disposable test tubes. For each sample tested you will require five test tubes, plus one for control.

5.1 Rehydrate the bacteria in Bottle B with the solution in Bottle C. Keep both bottles cold before mixing. Remove the seals and stoppers from the two bottles and immediately transfer the medium from Bottle C to Bottle B. Mix well by gently shaking. Leave at room temperature for fifteen minutes.

5.2 Transfer 1.0 ml (or up to mid-way between the red and blue lines on the plastic pipet) of bacterial suspension from Bottle B into 9.3 ml of Reaction Mixture in Bottle A.

5.3 Keep the bacteria reaction-mixture in (Bottle A) at room temperature for about ten minutes before dispensing 1.0 ml (or up to the blue line on the plastic pipet) of the bacteria reaction mixture into the first tube, and 0.5 ml (or up to the red line on the plastic pipet) into the remaining tubes (two to five).

5.4 Place 0.5 g of wet sediment sample into the first tube and mix thoroughly.

5.5 Transfer 0.5 ml (or up to the red line on the plastic pipet) of the bacteria-sediment mixture into the second tube, second to third, third to fourth and fourth to fifth.

5.6 After mixing tube 5, 0.5 ml (or up to the red line on the plastic pipet) of mixture is discarded.

5.7 Before each transfer, the bacteria-sediment mixture is hand-shaken vigorously or vortex.

Make sure to transfer the suspended portion of solid particles. Cut off the pipet tips if necessary.

5.8 Place 0.5 ml (or up to the red line on the plastic pipet) of bacterial suspension into the control tube.

5.9 All tubes are then incubated for two hours at 37°C.

5.10 After the two-hour incubation, hand-mix vigorously each tube again immediately before transferring a small drop from each tube onto the chromogenic pad (see Figure 2).

For liquids and light solids (e.g., sand), continue with Steps 5.11 to 5.15, as follows:

5.11 Cover the petri dishes and incubate at 37°C for one hour, or until a visible blue colour develops in the control (central) blot.

5.12 After the incubation, wash off the solids from the pad with water using a squirt bottle.

5.13 Check the reverse side of each transfer spot for a blue colour development.

5.14 If the sample is toxic, no blue colour will develop and if the sample is non-toxic, a blue colour will develop under the sample spot.

For all other solids, continue with Steps 5.11 to 5.14, as follows:

5.11 Cover the petri dishes and incubate at 37°C overnight (or ≥ ten hours).

5.12 After the incubation, was off the solids from the pad with water using a squirt bottle.

5.13 Check the reverse side of each transfer spot for a blue colour development.

5.14 If the sample is toxic, no blue colour will develop and if the sample is non-toxic, a blue colour will develop under the sample spot.

6. ANALYSIS OF THE RESULTS

Check the colour density appearing in the reverse side of each transfer spot on the pad. High toxicant concentrations may not have any colour due to toxicity. As the material is diluted out, toxicity is reduced and colour density will increase up to the maximal level of the controls.

Results can be recorded by determining the highest sample concentration which gave complete inhibition, i.e., no colour development. This is the sample's Effective Concentration 100 (EC_{100}) value expressed in percent units. For example, if no colour is developed in the second dilution, but some colour develops in the third dilution, the EC_{100} value is 25%.

7. REFERENCES

Kwan, K.K. 1993. Direct solid phase toxicity testing procedure. Environ. Toxicol. Wat. Qual. 8: 345-350.

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Reinhartz, A., L. Lampert, M. Herzberg and F. Fish. 1987. A new, short-term, sensitive bacterial assay kit for the detection of toxicants. Toxic. Assess. 2: 193-206.