Lead Test Kit Protocols

Experiment: LeadTrak Fast Column Extraction

Materials:

- LeadTrak Reagent Set
- 150 mL beakers
- 250 mL beakers
- 15 mL conical tubes
- 50 mL conical tubes
- 1.5 mL microcentrifuge tubes
- Clamp and clamp holder
- 25 mL graduated cylinder
- 100 mL graduated cylinder
- 1 mL droppers
- Sample Cells

Methods:

Part I: Creating a Standard Curve

1.) Fill graduated cylinder with 100 mL of the distilled H_2O. Pour sample into a 250 mL beaker.

2.) Using 1mL dropper, add 1.0 mL of pPb-1 Acid Preservative Solution to the sample and swirl to mix.

3.) Start instrument timer. A 2 minute reaction will occur.

4.) When the timer expires, use a second dropper to add 2.0 mL of pPb-2 Fixer Solution. Swirl to mix.

5.) Mount new Fast Column Extractor in a ring stand. Place a 150 mL beaker under the extractor.

6.) Soak the cotton plug with DI water and compress it with the plunger. Remove the plunger. If the cotton plug moves up the column, push it back to the bottom with a blunt rod.

7.) Pour the prepared sample slowly into the center of the Column Extractor. Wait for the sample to flow through.

8.) After the flow has stopped, fully compress the absorbent pad in the Extractor with the plunger. Discard the contents of the beaker. Slowly withdraw the plunger from the extractor.

9.) Place clean, dry 150mL beaker under the Extractor. Using a 25 mL graduated cylinder, add 25 mL of pBb-3 Eluant Solution to the Extractor. Allow the Eluant Solution to drip slowly from the extractor.

10.) Using a 1 mL dropper, add 1.0 mL of the pBb-4 Neutralizer solution to the beaker. Swirl thoroughly to mix and proceed immediately to the following step.

11.) Add the contents of the pBb-5 indicator Powder Pillow to the beaker and swirl thoroughly to mix.

12.) Pour 10 mL of the solution into a 15 mL conical tube. Add 3 drops of pBb-6 Decolorizer Solution. Invert the tube to mix. Label this tube “B”.

13.) Pour the remaining contents from step 11 into a separate 15 mL conical tube. Label this tube A.

14.) Set the spectrophotometer to OD477. Using the sample B, blank the reading.

15.) Measure the absorbance at OD477 for the sample A and record the reading.

16.) Repeat steps 1-15 with samples with known lead concentrations of 50 ppb, 100 ppb, and 150 ppb by using 0.5 mL, 1 mL, and 1.5 mL of the lead standard provided in the kit and 99.5 mL, 99 mL, and 88.5 mL of distilled H_2O, respectively.

17.) Plot the absorbance values to create a standard curve that maps lead concentration to measured absorbance.

Part II: Testing a Bacterial Sample

1.) Start a culture by picking 1-5 colonies of desired bacterial and adding them to 10 mL of media in a 50 mL conical tube.

2.) Incubate at 37ºC and 240 RPMs. Be sure that the cap is loosely screwed and secured with tape.

3.) 14-18 hours later, measure the OD600 of the culture.

4.) Calculate the volume of original culture needed to dilute the culture to an OD600 of 0.1 into a total volume of 20 mL using fresh LB.

5.) Calculate the volume of fresh LB needed to dilute the culture to an OD600 of 0.1 into a total volume of 20 mL.

6.) Add 7.5 uL of a 100,000 ppm lead stock to the fresh LB.

7.) Before adding the original sample to the fresh LB plus lead solution, take a 1 mL sample and add it to a 1.5 mL microcentrifuge tube.

8.) Spin down the sample in a table-top centrifuge at high speed for 5 min.

9.) Add the calculated volume of the original culture to the fresh LB plus lead solution.

10.) Incubate at 37ºC and 240 RPMs. Be sure that the cap is loosely screwed and secured with tape.

11.) Fill graduated cylinder with 99.9 mL of the distilled H_2O. Pour into a 250 mL beaker.

12.) Once the centrifuge cycle in complete, add 100 uL of sample to the beaker.

13.) Using 1mL dropper, add 1.0 mL of pPb-1 Acid Preservative Solution to the sample and swirl to mix.

14.) Start instrument timer. A 2 minute reaction will occur.

15.) When the timer expires, use a second dropper to add 2.0 mL of pPb-2 Fixer Solution. Swirl to mix.

16.) Mount new Fast Column Extractor in a ring stand. Place a 150 mL beaker under the extractor.

17.) Soak the cotton plug with DI water and compress it with the plunger. Remove the plunger. If the cotton plug moves up the column, push it back to the bottom with a blunt rod.

18.) Pour the prepared sample slowly into the center of the Column Extractor. Wait for the sample to flow through.

19.) After the flow has stopped, fully compress the absorbent pad in the Extractor with the plunger. Discard the contents of the beaker. Slowly withdraw the plunger from the extractor.

20.) Place clean, dry 150mL beaker under the Extractor. Using a 25 mL graduated cylinder, add 25 mL of pBb-3 Eluant Solution to the Extractor. Allow the Eluant Solution to drip slowly from the extractor.

21.) Using a 1 mL dropper, add 1.0 mL of the pBb-4 Neutralizer solution to the beaker. Swirl thoroughly to mix and proceed immediately to the following step.

22.) Add the contents of the pBb-5 indicator Powder Pillow to the beaker and swirl thoroughly to mix.

23.) Pour 10 mL of the solution into a 15 mL conical tube. Add 3 drops of pBb-6 Decolorizer Solution. Invert the tube to mix. Label this tube “B”.

24.) Pour the remaining contents from step 11 into a separate 15 mL conical tube. Label this tube A.

25.) Set the spectrophotometer to OD477. Using the sample B, blank the reading.

26.) Measure the absorbance at OD477 for the sample A and record the reading. This is the lead concentration of the sample at T0.

27.) At T1, T4, and T24, take a 1 mL sample and add it to a 1.5 mL microcentrifuge tube.

28.) For each sample, spin down the sample in a table-top centrifuge at high speed for 5 min and repeat steps 11-26.