The Dirt Detective
User Instructions

An easy at home kit to test the phosphate and copper levels in your soil.
For more information you can visit our project website: https://2022.igem.wiki/wlc-milwaukee/description
This kit was made by the WLC iGEM team in 2022.

This kit contains the supplies to run x number of tests.:

Item	**Quantity**
Bacteria	30 ml/test
Nitrocefin	2.5 mg/ml
50 ml conical tubes for sample collection	1 tube/sample
15 ml tubes for sample testing	7 tubes/test
Tubes with 4ml and 2 ml markings	7 tubes/test
Filter paper	1 piece/test
10mM Tris buffer pH 7.5	20 ml/test
Stock phosphate or copper solutions	4 ml/test
Bottle containing nitrocefin solution with dropper	1 ml
25 ml measuring device	1
Funnel	1
10 ml syringe	1/sample
0.45 micron syringe filter	1/sample

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Step by Step Instructions For Use

Wash your hands
To avoid contamination and decrease the chance of getting false results, we recommend you to wash your hands and make sure your workspace is as clean as possible. Also, wash your hands after using the kit. While all the chemicals and organisms used in this kit are safe, it is best to be completely safe and make sure you do not transfer these components anywhere else.

Preparation
One test equals six controls and at least one sample. If testing multiple samples at the same time on the same day, you can use one set of six control tubes. If you are performing tests at different times during the day or on different days, set up a new set of control tubes for each time or day.

On the outside of the kit box, locate the perforated circles. Punch out the circles. This will allow you to use the box as a rack to hold the tubes upright while you use the kit.

Step 1 (day before testing)

Preparing the bacteria
In the kit, you will find a bottle containing Lysogeny broth (LB) broth and a 50 ml tube labeled "*E. coli* - phosphate" or "*E. coli* copper". In the "*E. coli*" tubes, there is lyophilized (dried) bacteria. Approximately 24 hours prior to testing soil samples, pour LB broth into the tube containing *E. coli* up to the 30 ml line on the tube. Close the top on the tube tightly and allow the tube to sit at room temperature (approximately 22-25 degrees °C) for 24 hours.

Step 2

Collecting a soil sample
Use a soil sample probe (or other preferred method) to obtain soil samples from different areas of the desired testing region. Add soil (loosely-packed) to a sterile 50 ml conical tube up to the 40 ml line on the tube. Prepare a new tube for each soil sample. To each soil sample use the 25 ml measuring device to measure 25 ml of the 10 mM Tris pH 7.5 buffer and add to each soil sample. Tightly close the cap on the tube and shake vigorously. Allow the tube to sit at room temperature for one hour.

Step 3

Filter sample
For each sample, open a sterile 15 ml conical tube and place a funnel in the tube. Fold a circular piece of filter paper twice and open it so that the cone shape of the filter paper fits in the funnel. After the soil sample (with buffer) has sat for one hour, pour it slowly into the filter paper cone in the funnel. Liquid will slowly go through the filter paper and collect in the 15 ml conical tube. When 5 ml of liquid has collected in the 15 ml conical tube, remove the funnel and throw away the filter paper with soil.

Screw the 0.45 micron syringe filter on to the end of the syringe. Remove the plunger from the syringe and pour the 5 ml from the 15 ml conical tube into the syringe. Put the plunger in the syringe and place the end of

the syringe filter in a new 15 ml conical tube. Add pressure to the plunger so the solution passes through the syringe filter into the new 15 ml conical tube. This is the solution you will use to test for nutrient concentrations.

Step 4

Preparing the *E. coli*

Check the tube containing the *E. coli* and LB buffer that you allowed to sit at room temperature for approximately 24 hours. While the liquid should have been mostly clear the previous day, now it should be more cloudy. This is due to growth of the bacteria.

Pour this culture into the tubes with the 4 and 2 ml markings on them. Use six tubes for the control tubes and one tube for each sample you are testing at that time. Centrifuge these tubes for 5 minutes **(This centrifugation is a step we have not figured out for a person to use this kit at home)**. Pour the liquid from the tubes. There should be a whitish spot at the bottom of the tubes which is the bacteria. Add the 10 mM Tris pH 7.5 buffer up to the 2 ml marking on the tube. Shake the tube until the white spot is no longer present on the bottom of the tube. This puts the bacteria back in the solution.

Step 5

Preparing the test

On four of the six control tubes, label them 1 mM phosphate, 0.1 mM phosphate, 0.01 mM phosphate, or 0.001 mM phosphate. If you are testing for copper instead of phosphate, label with the same concentrations except “copper” instead of “phosphate”. On the fifth control tube, label it “No nitrocefin” and on the sixth control tube, label it “No phosphate”. Then make sure your samples are labeled so you can tell them apart.

To each of the control tubes labeled as phosphate concentrations, add 0.5 ml of the appropriate stock solution. To the tube labeled “no nitrocefin”, add 0.5 ml of the 1 mM phosphate solution. To the tube labeled “no phosphate”, add 0.5 ml 10 mM Tris pH 7.5 buffer.

After the solutions are added, add one drop of the nitrocefin solution from the nitrocefin solution bottle to all of the tubes except for the tube labeled “no nitrocefin”.

Shake the tubes briefly by hand to mix everything and allow the tubes to sit at room temperature for one hour.

Step 6

Analyzing the results

After one hour the results are ready to be examined. The four tubes that received the phosphate or copper solutions should have turned varying degrees of red color. The brighter the color red, the higher the concentration of phosphate or copper will be in solution. The “no nitrocefin” tube should not have turned red. The “no phosphate” or “no copper” tubes may have some red color but it should not be as strong as the control tubes that received the phosphate or copper solutions.

Observe the sample tubes that you prepared. Compare the amount of red color in those tubes to the color in the tubes that received the phosphate or copper solutions. The tube control tube to which your sample tube most closely matches will determine the concentration of phosphate or copper in your sample solution which should provide the concentration of phosphate or copper in the soil sample.

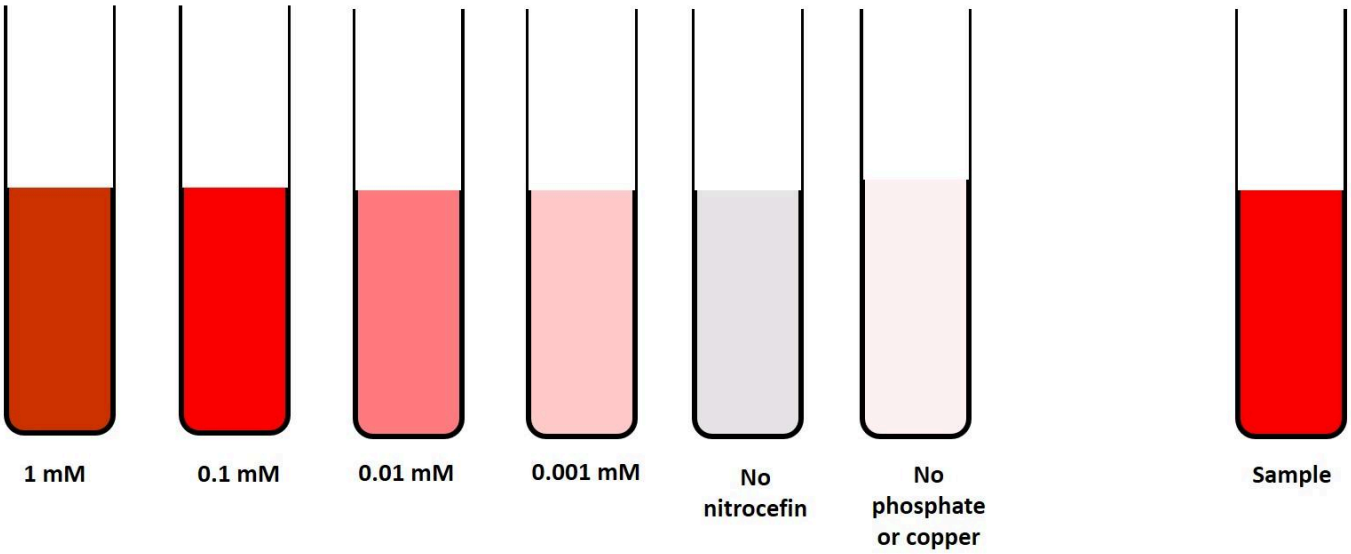
If there is no color in your sample tubes, that could mean that there is very little phosphate or copper in the soil sample or there was a problem in setting up the experiment. Set up the experiment again or use another testing method to confirm the result.

Step 7

Disposal
Upon completion of the experiment, all solutions can be disposed of in a sink with water running and all tubes and other materials can be disposed of in regular trash. No tubes, filter paper, syringe, or syringe filter should be reused but the funnel can be cleaned and reused.

Results

If the test worked ideally, the tubes would look similar to the diagram below.



These results show a decrease in red color as the concentration of either phosphate or copper solution decreases. The “no nitrocefin” tube does not have a red color and the “no phosphate” or “no copper” tube only shows a slight red color. The tube that was prepared from the soil sample shows a color that falls within the range of colors seen in the control tubes. Since the color of the sample is most similar to the 0.1 mM control tube, it would be concluded that this is the concentration of phosphate or copper in the soil sample.

Fact Sheet: The Biology of Our Project

How does this kit work?

Step 1
In this step the dried *E. coli* is “woken up” and allowed to grow. The LB broth provides nutrients to allow the bacteria to grow and multiply. This is important because the *E. coli* cells contain the genetic components that will sense the specific nutrient and produce a protein that will cause the solution to turn red when the nitrocefin is added.

Step 2
A soil sample is acquired from any location. It could be a surface sample or a sample at specific depths. Each sample will be unique. The soil is acquired in a conical tube which will allow a relatively consistent amount of soil to be collected. The Tris buffer is added to get some of the nutrient out of the soil and into solution. Phosphate and calcium are capable of dissolving in the buffer but may take some time to dissolve so that is why the sample is allowed to sit for one hour.

Step 3
At this point some of the nutrients should have dissolved in the buffer. We need to reclaim the buffer to test for the nutrient concentration. It would be difficult to observe the red color we will see in our later results if the soil was still in the sample so we need to filter the buffer out of the soil. Thus, we set up two filtering methods. The first step of putting the filter paper in the funnel and pouring the soil and buffer solution in it will filter out the largest soil components but there are still some contaminants in the solution. This requires a filter that gets rid of small particles. The 0.45 micron syringe filter gets rid of any particles larger than 0.45 micrometers in diameter. This removes particles as small as bacteria. After the filtering takes place the solution is ready to be used for the testing.

Step 4
Now that the soil sample has been prepared, it is necessary to prepare the *E. coli* strains that will detect the nutrient and produce an enzyme that will give off a red color when the nitrocefin substrate is added. The *E. coli* that tests for phosphate concentration has a gene for the enzyme Beta-lactamase that is controlled by the concentration of phosphate present in the solution that is added. So, when different concentrations of phosphate are provided, varying amounts of Beta-lactamase is produced. When the drop of nitrocefin is added, Beta-lactamase will cleave this molecule and this will cause a red color to be produced. With more Beta-lactamase, there will be more red color.

The *E. coli* strain that measures copper is very similar. It will produce varying amounts of Beta-lactamase depending on the amount of copper that is present in the solution. The stock solutions containing different concentrations of phosphate or copper are standards that will be used to compare the samples.

Step 5
In step 5, the experiments using the controls containing the stock concentrations, no nitrocefin, and no phosphate/copper are set up. The experiments with the samples prepared from the soil are also set up. All the components are added together and allowed to interact for one hour. This allows the Beta-lactamase to be produced based on the amount of phosphate or copper present and the Beta-lactamase will cleave the nitrocefin to produce the red color.

Step 6
Now that the solutions have been allowed to sit for one hour, they can be compared to determine how much nutrient is in the soil. Comparing the color of the sample tube to the control tubes will give the concentration of the nutrient.

Step 7
All solutions and organisms are safe but should be handled carefully. The *E. coli* should remain contained in the tubes and thrown away. All solutions and buffers can be poured down the sink if needed or just thrown away in normal trash.

Safety Information

This kit contains live E. coli, however it is a non-pathogenic lab strain of E. coli, specifically DH5-alpha. This strain is a commonly used lab strain and does not carry any virulence factors associated with wild-type, pathogenic E. coli.

In the testing and making of The Dirt Detective kit, we modified an organism’s genome using synthetic biology. We did not, at any point, modify the E. coli in any way that would affect the virulence factors or pathogenicity.