Making Milk Curdle with Pineapple Enzymes


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Experimental Procedure

- Resistors are marked with colored bands. These colors do matter. Make sure you pick the right resistors for each step according to the markings.
- It matters which direction some of the components are facing. Make sure you read the slideshow captions for any special notes about inserting each part.
- This section only shows you how to assemble the circuit. For a detailed explanation of how the circuit works, see the Help (help) section.

Slideshow with step-by-step instructions viewable online.
<table>
<thead>
<tr>
<th>Part name</th>
<th>Picture</th>
<th>Breadboard Symbol</th>
<th>Location</th>
</tr>
</thead>
</table>
| 9 V battery        | ![Image of 9 V battery](image1.png) | ![Breadboard symbol](image2.png) | Red wire to (+) bus  
Black wire to (-) bus |
| Photoresistor      | ![Image of Photoresistor](image3.png) | ![Breadboard symbol](image4.png) | R24, R26                |
| White LED          | ![Image of White LED](image5.png) | ![Breadboard symbol](image6.png) | Long lead to D17  
Short lead to D16       |
| Jumper wires (3)   | ![Image of Jumper wires](image7.png) | ![Breadboard symbol](image8.png) | J26 to multimeter  
J24 to multimeter  
A17 to (+) bus         |
| 220 Ω resistor     | ![Image of 220 Ω resistor](image9.png) | ![Breadboard symbol](image10.png) | ±16 to (-) bus          |

**Table 1. Components for the spectrophotometer circuit (Image credits: Jameco and Fritzing).**

### Testing the Turbidity Meter

After you have finished building your circuit, testing the turbidity meter is necessary to ensure that all the electronic components are connected correctly and your device works as expected. Note: Shiny light will cause problems and may result in fluctuating data. Perform the readings in a dimly lit room if shiny light is a problem and make sure to always place the cuvettes into your device the same way. If the sides of your cuvettes are not all clear, but two of them are grooved or frosted, make sure that the clear side is always facing toward the LED and the frosted or grooved side is facing toward the photoresistor. Place a heavy bowl or cardboard box upside-down over your breadboard during measurement to block out surrounding light. If you get fluctuating data, make sure your cuvettes are free of smears or dirt on the outside and there are no air bubbles sticking to the sides.

1. Cut a small piece of dark construction paper and roll it into a tube that just fits on the head of the LED. The tube length should be about 2 cm. Place the tube on the head of the LED and attach it with some tape so it is not able to slide off.
2. Place an empty cuvette upside-down over the photoresistor. Make sure that for this cuvette, the clear side faces toward the front of the photoresistor. Bend the photoresistor leads as needed to fit underneath the cuvette. Use tape to hold the cuvette over the photoresistor in place, but do not block the light path.
3. The light path from the LED and the tube should be exactly aligned with the height of the photoresistor, as shown in Figure 3. Bend the wires on the LED and photoresistor for adjustment, if needed.
4. Set up the multimeter to measure the resistance of the photoresistor.
   a. Plug the black multimeter probe into the port labeled COM.
   b. Plug the red multimeter probe into the port labeled VΩmA.
   c. Turn the dial setting to 200 kΩ.
   d. Turn the power switch ON.
   e. Make sure you remembered to use the alligator clips to attach the multimeter probes to the jumper wires connected to the photoresistor coming from J24 and J26.
5. As you connected the jumper wire from A1 to the power (+) bus already, your LED should still be turned on.
6. Cover the circuit (but not the multimeter) with a dark bowl or a cardboard box to block ambient light.
7. Read the resistance across the photoresistor and record it in your lab notebook.
   a. Note the units of the resistance. A "K" indicates kilo-ohms (kΩ).
   b. If your multimeter screen displays a "1.", that means the resistance is too high for the dial setting. Turn the dial up to the next highest range (for example, from 200 k to 2000 k) and check again.
   c. If this is your first time using a multimeter, refer to the Science Buddies resource How to Use a Multimeter [link], specifically the section How do I measure resistance? [link], to learn more.
8. Remove the bowl and turn off the LED by removing the jumper wire from the power (+) bus.
9. Cover the circuit with the bowl again. In the dark, the resistance should be in the mega-ohm range. Remember that you may need to adjust the dial setting to get a measurement. Record the resistance in your lab notebook.
10. Remove the bowl and turn off the multimeter to conserve battery power.

Preparing and Measuring Your Test Samples

In this step, you will measure a range of test solutions on your turbidity meter to demonstrate how different amounts of particles or colloids present in a sample result in different light scattering.

1. Label six clear small plastic cups 1–6 with the masking tape and permanent marker.
2. You will make a series of 1:10 dilutions with the following contents, as shown in Figure 4:
   a. Water
   b. 100 percent fat-free milk
   c. 1/10 dilution
   d. 1/100 dilution
   e. 1/1,000 dilution
   f. 1/10,000 dilution

Figure 3. The light path of the LED should be directly aligned with the height of the photoresistor.
3. Put 10 mL of water in cup #1 and 10 mL of fat-free milk in cup #2.
4. Put 9 mL of water into cup #3. Add 1 mL of fat-free milk. Stir with a clean spoon.
5. Put 9 mL of water into cup #4. Add 1 mL from cup #3. Stir with a clean spoon.
6. Repeat the 10-fold dilutions for cups #5 and #6.
7. Transfer the water and all other solutions into six clean and labeled cuvettes. Use an eye dropper or a transfer pipette, or pour carefully. Note: The cuvettes hold approximately 3 mL of solution. Also, make sure to label the cuvettes at the very top so as not to cover the light path with your writing.
8. Now you are ready to measure scattered light from each of your test samples. Move the circuit and the cuvettes to a work area with dim light. There should be just enough light for you to work and to read the multimeter. You could use a nightlight or a red LED flashlight as a work light.
9. Attach the red and black multimeter probes to the red and black wires in contact with the photoresistor (coming from J24 and J26) using the cables with the alligator clips, if they are not yet connected.
10. Set the multimeter to read resistance again. Remember that you might have to adjust the range as you take different readings.
11. First, place your blank sample with only water in the corner between the LED and the photoresistor, as shown in Figure 5. Remember to face the clear side of the cuvette with your sample solution toward the LED and the frosted or grooved side toward the photoresistor.

Figure 5. Setup of the turbidity meter for measuring your test solutions and milk samples. Note that in these pictures the LED is switched on, but the setup is not covered with a bowl yet.

12. Plug in the wire to turn on the LED and cover the breadboard with the bowl. Read the resistance on the multimeter and record the data in your lab notebook.
13. Remove the blank cuvette and replace it with the cuvette containing the next solution, starting with the lowest dilution (1:10,000). Cover the breadboard again with the bowl and write down the resistance for this solution. Continue the measurements for each of your five milk solutions.
14. Repeat steps 11–13 with the entire set of standards, including the blank, two more times.
15. Make a data table in your lab notebook, showing the different dilution levels together with all three recorded resistance measurements for each solution. The resistance should be higher as the solutions get less concentrated.

Tracking Milk Curdling

Now that you have a working turbidity meter, you will use it to track the curdling of milk in the presence of the proteolytic enzyme bromelain from pineapple juice. As bromelain causes the light-scattering particles in the milk to coagulate, the milk becomes more transparent and therefore scatters less light.

1. Label three clean cuvettes 1–3 with a permanent marker.
2. Make 50 mL of 10 percent milk by adding 5 mL of milk to 45 mL of water.
3. Add 3 mL of the 10 percent milk to each cuvette.
4. Squeeze 20 mL of fresh pineapple juice into a clean container.
   a. To get the pineapple juice, take a fresh pineapple, cut off the end, and grate the flesh. Place the grated fruit in a piece of cheesecloth and squeeze


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over a clean container.

b. Any fresh pineapple fruit will work, as will frozen pineapple that has been thawed at room temperature. Canned pineapple or refrigerated pineapple juice does not work, as they are heat treated and heat destroys enzymes.

5. Transfer 10 mL of the juice into a small, microwave-safe container. Microwave the pineapple juice for about 20 seconds, or as long as necessary to get the juice to boil for 3–5 seconds. Heat will destroy enzymes, but will not affect most chemicals. The point is to show that enzymatic activity, which is heat-sensitive, is what causes the milk to clear.

6. Place the first cuvette with the 10 percent milk in your turbidity meter with the clear side facing toward the LED and the frosted or grooved side toward the photosensor. Make sure the device is ready for taking measurements, switching on the LED and setting the multimeter to measure resistance.

7. Get your stopwatch ready and add one or two drops of fresh, unheated pineapple juice to the cuvette using an eye dropper or a transfer pipette. Immediately start the stopwatch once you add the pineapple juice. Note. You may need to change the amount of juice you add, depending on how fast the reaction occurs under your experimental conditions. Add less pineapple juice to get a slower reaction, and more pineapple juice for a faster one.

8. Place the bowl over the turbidity meter to block surrounding light and measure the amount of scattered light by recording the resistance from the multimeter every 2 minutes for 20 minutes.

9. Once the 20 minutes are over, remove the cuvette from your turbidity meter and replace it with the second cuvette with 10 percent milk. Repeat the experiment, but this time add the same amount (one or two drops) of the heated, inactive pineapple juice to the cuvette. Again, start the stopwatch once you add the pineapple juice and record the resistance readings every 2 minutes for 20 minutes.

10. In the next step, use the third cuvette with 10 percent milk and repeat the experiment with no additions to the milk solution. Make sure to record the resistance readings from the multimeter every 2 minutes for 20 minutes.

11. Finally, repeat each experiment (10 percent milk with the addition of fresh pineapple juice, heated pineapple juice, and no additions), following steps 6–10 two more times.

Analyzing Your Data

1. Open a spreadsheet and enter your data (the different milk dilutions and the resistance values of these solutions). Calculate the average of your three resistance readings for each test sample.

2. Graph the average resistance of your three readings (scattered light) on the y-axis versus the dilution of the milk on the x-axis. You will notice that the relationship between measured intensity of scattered light (or the resistance) and milk concentration is nonlinear. You can linearize the data by performing a base-10 logarithmic transformation of your data. Do not include the water sample in these calculations. Now simply graph the average resistance data on the y-axis and the log10 of the milk dilutions on the x-axis. You should get a linear curve this time.

3. You can add a trend line to the linearized data and display its equation and its correlation factor R^2 to show the correlation between light scattering and the milk (or fat droplet and casein micelle) concentration. How does the light scattering (resistance) change when you dilute the milk?

4. For each of your test samples (10 percent milk with the addition of fresh pineapple juice, heated pineapple juice, and no additions), enter your resistance data into the spreadsheet and calculate the average of your three readings for each of them.

5. Graph the average resistance (scattered light) versus time in minutes for each of your samples. If you want, you can use your milk dilution vs. resistance curve to determine the milk dilution factor before and after coagulation for each of your samples. Remember, that the results from your calculations will be the log(milk dilution factor). To get the real milk dilution factor, you have to convert your result from log(milk dilution factor) to milk dilution factor by taking your calculated result from your curve and raise 10 to that number. How does the pineapple enzyme change the light-scattering behavior of your milk samples? Do you see a difference between your samples treated with fresh versus heat-inactivated pineapple juice?

Frequently Asked Questions (FAQ)