Experimental Procedure

Testing the Glucose Strips

In this part of the science project, you will create controls, which are samples with known ingredients that should give clear, expected results. You will do this to make sure that the glucose test strips are working properly. If the test strips are not working properly, then the rest of this experiment will not be valid. The positive controls will contain different concentrations of glucose. The negative control will be a sample without glucose.

1. First, make the positive controls using water and the glucose powder. To do this, make a dilution series using sequential twofold dilutions to create the following concentrations: 2%, 1%, 0.5%, 0.25%, 0.125%, and 0.0625%.
   a. Label six cups: 2%, 1%, 0.5%, 0.25%, 0.125%, and 0.0625%.
   b. Add 4 grams (g) of glucose to 200 mL of water in the cup labeled 2% and stir until the glucose dissolves.
   c. Optional: Add 2–5 drops of food color to the 2% glucose solution. The color does not matter. Note: The food coloring will allow you to keep track of your dilution levels as the color of each dilution will get less intense. It does not interfere with the glucose measurements.
   d. Add 100 mL of water to the other five cups.
   e. Measure 100 mL of the 2% solution and add it to the cup labeled 1% to make a 1% solution. Stir well.
   f. Measure 100 mL of the 1% solution and add it to the cup labeled 0.5% to make a 0.5% solution. Stir well.
   i. Between each dilution, make sure to rinse and shake the excess water from the graduated cylinder or container you are using to transfer the 100 mL volumes. Also, use a clean stirrer.
   g. Repeat this process for the remaining dilutions.
   i. When you are done, each cup should have 100 mL of liquid, except for the 0.0625% solution, which should have 200 mL.
2. Fill an extra cup with 100 mL water. Do not add any of the glucose solutions to it. Label it 0%. This will be your negative control.
3. If you used food coloring for your dilution series, you should now have seven cups that look similar to the ones in Figure 2.

![Figure 2](https://www.sciencebuddies.org/science-fair-projects/project-ideas/FoodSci_p073/cooking-food-science/lactose-sucrose-and-glucose-how-many-sugars-are-in-your-smoothie/figure2.jpg)

Figure 2. If you used food coloring (in this picture red food coloring was used), the glucose dilution series should look like the ones in this picture (arranged by most concentrated to least, from left to right). Each cup should have 100 mL of liquid, except for the 0.0625% solution, which should have 200 mL. A seventh cup, serving as the negative control, should only contain water (on the far right in this picture).
4. Dip a test strip into each of the seven cups, one at a time. After 1–2 seconds, remove the test strips from each solution and watch them for 30 seconds (which should be the time recommended in the test strip instructions). Then match the color of the glucose marker on the test strip to the color on the bottle shown in Figure 3. Do the colors match what you would expect? Write down your observations in your lab notebook. **Note:** For high glucose concentrations, it might take up to 60 seconds until the color matches the actual concentration. Therefore it is recommended that you dilute your samples once they approach a glucose concentration of 1%.

a. See the Technical Note for guidance on matching the color of the glucose test strips to the color on the bottle.
b. If the color changes to the maximum range (2%) before 30 seconds, list it as greater than 2% (">2"). You do not need to perform a dilution.
c. If you do not have a clear color change for any of the positive control solutions with a concentration greater than 0.0625%, repeat the procedure. If the second time it is still problematic, you might have to buy new test strips. It is ok to have a slightly lower reading for the pure glucose solutions. Remember, these test strips were designed for measuring low concentrations of glucose in a urine sample, so the results might be slightly different for pure glucose solutions. If the test strips for the glucose solutions at 30 seconds are more than one color off from what it is expected to be (for example, if the 1% solution reads less than 0.5% or the 0.25% solution reads greater than 0.5%), you could adjust the readout time accordingly (for example to 60 seconds). However, you have to make sure that throughout the experiment, you keep the same readout time for all of your samples.
d. **Tip:** If you would like additional help with reading the glucose test strips, check out the Frequently Asked Questions (FAQ) for this science project.

**Technical Note**

When matching the color of a glucose test strip to a color on the bottle, keep these helpful tips in mind:

- The colors on the bottle will not exactly correspond to the percent glucose solutions you made. There will probably be colors for 0% ("Negative"), 0.1%, 0.25%, 0.5%, 1% and 2% glucose solutions, as shown in Figure 3.
- Some test strip colors may fall between two of the colors on the bottle; for example, between 0.5% and 1%. If this happens, write down the two numbers in your lab notebook and calculate their average.
- If the color changes to the maximum range (2%) before 30 seconds, list it as greater than 2% (">2"). Depending on where this happens in the Procedure, you may need to then perform a 1:10 dilution and re-test the sample. You will get more accurate results if you start diluting your samples once the glucose concentration is getting close to 1%. There are two ways in which you may perform a 1:10 dilution, and the preferred way will be specified in the text:
  - Use a transfer pipette to add nine drops of water and one drop of the test solution on a bottle cap. Rinse the transfer pipette in between each sample.
  - Mix 1/2 teaspoon (tsp.) (2.5 mL) of the sample with 22.5 mL water to make a 1:10 dilution. (Note: You will only test 15 mL of this dilution.)

Remember that if the 1:10 dilution reading reports 1% glucose, then the glucose in the sample is really 10%, because it was diluted tenfold.

**Glucose**

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**Figure 3.** This is the color chart for glucose on the test strip bottle. After a glucose test strip is dipped in a glucose solution and removed, its color should change and match a color on its bottle (or be between two colors). The color on the bottle will indicate the percentage of glucose in the solution tested.

**Testing the Activity of Invertase and Lactase**
In this next part of the science project, you will test the activity of the invertase and lactase enzymes. It is important for you to do this step so that you know how long you should test your selected foods with the digestive enzymes. You will test the activity of the invertase enzyme by investigating how long it takes to turn a known amount of sucrose (in solution) into glucose. Similarly, you will test the activity of the lactase enzyme by investigating how long it takes to turn a certain amount of lactose (in solution) into glucose. When invertase is added to the sucrose solution, or when lactase is added to the lactose solution, the concentration of glucose should increase over time as the sugars are converted to glucose. However, after some amount of time, the concentration of glucose will appear to remain the same, or plateau. For an example, see Figure 4. Although the invertase may still be converting sucrose to glucose, it is doing it at an extremely reduced rate. (This is probably partly due to product inhibition, which is when the product of a reaction—glucose in this case—stops the enzyme from making more product.) In this section, you will determine how much time is needed for the invertase enzyme to convert the sucrose in a 10% solution, and how much time is needed for the lactase enzyme to convert the lactose in a 10% solution.

1. Make a solution containing 10% sucrose.
   a. Fill a cup with 60 mL of room-temperature water.
   b. Add 6 g of sucrose to the cup of water. Mix until all the sucrose dissolves.
   c. Put 15 mL (1 Tbsp) of this solution into a new cup.
      i. How many grams of sucrose are in 15 mL of the 10% solution?

2. In your lab notebook, make a data table in which to record your data. You will be taking glucose readings over time to see how much sucrose has been converted to glucose by the invertase enzyme.
   a. Starting at zero, plan on taking glucose readings every 5 minutes (min) for the first 30 min, and then every 10 min after that. Plan on taking readings for 90 min total.

3. Use a glucose test strip to determine the concentration of glucose in the sucrose solution, as you did in step 4 of the "Testing the Glucose Strips" section, above. Write your result in the data table in your lab notebook under "0 minutes."
   a. There should be 0% glucose in the sucrose solution.

4. Set a timer for 90 minutes or make sure a clock is nearby.

5. Get one of the bottles from the sugar metabolism kit that contains 1 g of powdered invertase and prepare it for the experiment. Note: You can prepare the invertase solution right before you start the experiment, however, the invertase works better if you prepare the solution one day in advance. You do not have to prepare both of the invertase bottles at the same time. You can keep the second bottle for future experiments or prepare it once you run out of invertase in this experiment.
   a. Fill the measuring cylinder with 25 mL of distilled water.
   b. Open the invertase bottle and add the water to the invertase powder.
   c. Then close the lid and shake the bottle until all the powder has dissolved.
   d. Important: Once rehydrated, the invertase solution needs to be kept in the refrigerator when not used.

6. Add 15 drops (about 0.75 mL) of invertase to the sucrose solution. Quickly mix the solution with a clean spoon.

7. Start the timer or write down the exact time in your lab notebook.

8. Use the glucose test strips to take glucose readings of the solution over time, as described in step 2 of this section.
   a. Write your results in the data table in your lab notebook.
   b. See the Technical Note, above, for guidance on matching the color of the glucose test strips to the color on the test strip bottle.
   c. Remember that the glucose readings are most accurate if you dilute your sample once the glucose concentrations reach about 1%. If the color changes to the maximum range (2%) before 30 seconds, list it as greater than 2% (">2%") and quickly perform a 1:10 dilution using 1 drop of your test solution (as described in the Technical Note on the third bullet point) to determine the actual percentage of glucose in the sample. Take a glucose reading of the 1:10 dilution.
   d. When the glucose reading has remained the same for at least 20 min (3 readings spaced 10 min apart), you can stop taking readings.

9. Now make a solution containing 10% lactose.
   a. Repeat step 1 using new, clean cups and lactose instead of sucrose.
   b. If you have trouble dissolving the lactose in the water, you could try using warmer water. Important: If you do this, be sure to let the solution cool to room temperature before testing it because temperature can affect enzyme activity.

10. Test the activity of the lactase enzyme using the lactose solution by repeating steps 2–8 of this section with the following changes:
    a. Substitute lactase for invertase.
    b. Use 15 mL of the lactose solution.
    c. You will only need 3 drops of the lactase enzyme (as opposed to the 15 drops you used with the invertase enzyme).
    d. Just like the invertase enzyme solution, be sure to store the lactase enzyme in the refrigerator immediately after you are done using it.
11. Optional: You may graph your results to more visually represent and analyze your data. To do this, make one graph for invertase and one graph for lactase. On both graphs, put the time on the x-axis and the glucose concentration on the y-axis.
   a. You should end up with graphs that roughly look similar to Figure 4, below.

   ![Graph of Glucose Concentration vs Time](image)

   **Figure 4.** The enzymes should initially convert the sugars to glucose at a roughly constant rate, creating a linear, or nearly straight, line. However, after some amount of time, the concentration of glucose appears to remain roughly the same, or plateau. Although the enzymes may still be converting some sugars to glucose, they are doing it at an extremely reduced rate. The red asterisk marks where the glucose concentration first leveled off. If you create graphs, they may only roughly match the line in this graph.

12. Look at your data and figure out at what time point the enzyme activity started to level off. (Figure this out separately for each enzyme.) For example, in Figure 4, above, this point is marked with a red asterisk.

13. For each enzyme, add 10 minutes onto the time you figured out in step 12 (further explained in 13.a.). Write these times down for each enzyme somewhere in your lab notebook. These are the times you will use to test your selected food items.
   a. At the plateau times you figured out in step 12, the enzymes should be mostly done converting the sugars to glucose, based on your data. You are adding an extra 10 minutes to this time just to make sure the enzymes are nearly completely done with this process.

**Testing Smoothie and/or Milkshake Ingredients for Glucose, Sucrose, and Lactose**

Now that you have determined how long it takes each enzyme to finish converting its sugar into glucose, you are ready to test your selected smoothie and/or milkshake ingredients. In this part of the science project, you will first test the glucose concentration of your selected foods. Then you will react each with invertase and, by determining how this changes their glucose concentrations, you will figure out how much sucrose was in the food. Lastly you will react the ingredients with lactase and, again by determining how this changes their glucose concentrations, you will figure out how much lactose was in the food.

1. In your lab notebook, make a data table for recording your data.
   a. For each food sample, you will take three glucose readings: one before adding any digestive enzymes, one after adding the invertase, and one after adding the lactase.
   b. You will use the times you determined for each enzyme from step 13 in the "Testing the Activity of Invertase and Lactase" section, above. Specifically, after measuring the glucose of each sample, you will add the invertase (to the same samples) and measure the glucose levels again after the time you determined for invertase has passed. Finally, you will add the lactase to each and measure the glucose levels after the time has passed for lactase.
   c. Test at least three different samples of each selected food. You may want to test these at different times because of the amount of time the tests require.
      i. Multiple trials help scientists make sure that their results are accurate and reproducible.

2. Label the cups with the ingredient samples you will test.
3. **Important:** Let all of the ingredient samples you will test come to room temperature before testing them. The activity of an enzyme is affected by temperature. It is important that all of the test foods are about the same temperature so that any differences you see in your data are not because the foods were differing temperatures.

4. You may need to specially prepare some of your ingredients for testing (**Important:** If you have to use water to make dilutions, make sure that the water is at room temperature):
   a. For testing viscous ingredients (like peanut butter or honey), dilute the samples 1:10 (or 10%) with water. Do this dilution using ½ tsp. of your sample (as described in the third bullet point of the Technical Note, above).
   b. For testing fruits, dilute the samples 1:5 (or 20%) with water and blend them in a blender. To do this you will want to weigh out the fruit to make a 1:5 dilution with enough water to blend.
      i. For example, you could use 47.4 g of a fruit and 237 mL (1 cup) of water. (47.4 g divided by 237 mL is 0.2, or 20%.)
   c. For testing powders (like malted milk powder or cocoa powder), dilute the powder 1:5 (20%) with water by dissolving 3 g in 15 mL (1 Tbsp.) water.

5. To each cup, add 15 mL (1 Tbsp.) of the ingredient (diluted or undiluted) that you will test. **Important:** If you have to use water to make dilutions, make sure that the water is at room temperature.

6. Use a glucose test strip to determine the concentration of glucose in each food sample, as you did in step 4 of the "Testing the Glucose Strips" section, above.
   a. See the Technical Note, above, for guidance on matching the color of the glucose test strips to the color on the bottle.
   b. If the color changes to the maximum range (2%) before 30 sec, list it as greater than 2% (">2%") and perform a 1:10 dilution using ½ tsp. of your sample (as described in the third bullet point of the Technical Note, above). Use the diluted sample for all tests.

7. Write the glucose concentration for each sample in your data table.

8. Set a timer for the time you determined for invertase (in step 13 of the previous section) or make sure a clock is nearby.

9. Add 15 drops (about 0.75 mL) of invertase to each ingredient sample. Quickly mix the samples. **Important:** Always be sure to use a clean, new spoon for each different solution you mix.
   a. Start only a few samples at a time so it is easier to manage them.
   b. Other than taking it out to quickly add to the samples, the rehydrated invertase solution should remain in the refrigerator.

10. Start the timer or write down the exact time in your lab notebook.

11. When the time you determined for the invertase enzyme has passed, use a glucose test strip to determine the concentration of glucose in each sample. Write this in your data table.
   a. See the Technical Note, above, for guidance on matching the color of the glucose test strips to the color on the bottle.
   b. If the color changes to the maximum range (2%) before 30 sec, list it as greater than 2% (">2%") and perform a 1:10 dilution using ½ tsp. of your sample (as described in the third bullet point of the Technical Note, above). Take a glucose reading of the 1:10 dilution.

12. After taking these glucose measurements, set a timer for the time you determined for lactase (in step 13 of the previous section) or have a clock nearby.

13. Then add 3 drops of lactase to each ingredient sample. Quickly mix the samples.
   a. Again, like the invertase, the lactase solution should remain in the refrigerator when it is not being used.

14. Start the timer or write down the exact time in your lab notebook.

15. When the time you determined for the lactase enzyme has passed, repeat step 11 to determine the glucose concentration in each sample.

16. If you did not test all of your ingredients at the same time (or three different samples of each ingredient), you will want to repeat this process (steps 2–15) until you have tested all of them.
   a. You may want to test these at different times because of the amount of time the tests require.

17. Graph your results. Make a bar graph and put the ingredient names of the samples on the x-axis and glucose concentration on the y-axis. Include all three glucose readings for each sample (before adding enzymes, after adding invertase, and after adding lactase); to do this, it may be easiest to cluster the three bars for each ingredient sample.
   a. **Tip:** If you diluted any of your samples when testing them, be sure to account for this in your graphs. The graphs should show the **undiluted** glucose concentrations.

**Analyzing Your Results and Testing Smoothie and/or Milkshake Recipes**

In this part of the science project, you will analyze your data, determine how much of the different sugars (glucose, sucrose, and lactose) are in each ingredient you tested, and come up with your own smoothie and/or milkshake recipes to give someone a high or low dose of glucose.
1. Look at the graph you made in step 17 of the “Testing Smoothie and/or Milkshake Ingredients for Glucose, Sucrose, and Lactose” section. Do the glucose readings you took before adding the digestive enzymes match what you would expect for these foods? What about after adding each enzyme?
   a. *Hint:* Remember that invertase converts sucrose to glucose, so if you saw more glucose after adding invertase, then the ingredient had sucrose in it. Likewise, since lactase converts lactose to glucose, if you saw more glucose after adding lactase, then the ingredient most likely had lactose in it.
   b. Which ingredients had the most glucose before adding the enzymes? Which had the least? Did any have no glucose?
   c. Based on any change in glucose levels after adding invertase, which ingredients had the highest sucrose concentration, and which had the lowest? Did any foods have no sucrose? Do your results match your predictions?
   d. What about the lactose levels? Based on any change in glucose levels after adding lactase, which ingredients had the highest and lowest lactose concentrations? Do your results match your predictions?
      i. *Note:* If you saw a slight increase in glucose after adding the lactase enzyme and this was completely unexpected, this could be because the sample had a large amount of sucrose and the invertase is still converting sucrose to glucose, though at an extremely reduced rate. To check for this, you could re-test the ingredient but this time use a greater dilution (at least 1:5).
   e. Overall, after adding both enzymes, which food ingredients had the largest amounts of glucose? Which had the least?

2. How do your results compare to the amount of sugar listed for these foods on their packaging?
   a. You can also take a look at the “Ingredients” to see what types of sugars might be in the foods. High-fructose corn syrup actually contains fructose and glucose.
   b. *Note:* If you want to determine the actual sucrose and lactose concentrations in the ingredients you tested, see the Make It Your Own section for how to do this. For this science project, only the relative concentrations are being analyzed.

3. How might this experiment be different from what takes place in the human digestive system? Do you think that even more glucose might have been made due to other chemical reactions taking place? *Hint:* Re-read the Introduction.

4. Knowing the sugar contents of different foods is particularly important for someone with diabetes. If someone has hypoglycemia and needs a fast glucose boost, which ingredients would you recommend he or she eat? Which ingredients would you recommend avoiding? Which ingredients would you recommend he or she uses in moderation, not only because they are high in glucose, but also because they are high in sucrose and/or lactose? Which foods may be safe for someone with diabetes to consume because they do not change blood glucose levels that much?

5. Think about your answers to step 4 of this section, and devise three different smoothie and/or milkshake recipes, as described below. Include at least two or three ingredients in each milkshake (at least one liquid and one additive). The ratio of ingredients you use is up to you! Then make your drinks and test them (only 15 mL each) as you did in the “Testing Smoothie and/or Milkshake Ingredients for Glucose, Sucrose, and Lactose” section above. Do your predictions match your results? Which drinks would be best for each type of medical condition or situation? For what medical situation would some of these drinks not be good to use?
   a. Make one milkshake or smoothie that should not change blood glucose levels much (if at all).
   b. Make a second milkshake or smoothie that should give someone a fast glucose boost.
   c. Make a third milkshake or smoothie that should not give much (if any) fast glucose boost, but will give a person a glucose boost over time.

**Frequently Asked Questions (FAQ)**