

## LACTASE ENZYME LAB

Lactase is an enzyme that works on lactose, a sugar. Lactose sugar is a disaccharide, two rings big. To digest it, you need to break it down into two pieces, two monosaccharides. Lactase enzyme allows you to do this. It reduces the amount of energy needed for the hydrolysis of the disaccharide into two monosaccharides.

Enzymes are proteins. Temperature can effect them. If you cook egg white protein, it changes from clear and runny to firm and white. It is denatured. Enzymes must fit with their substrate in order to work. If an enzyme is denatured, it cannot fit anymore. An enzyme that cannot fit, cannot do its job.

We will test the effect of temperature on how well lactase enzyme works. First, we must have a way to test whether the enzyme is working or not. We will use an indicator that changes color. TesTape is the indicator. It changes color in the presence of glucose, a monosaccharide.

**IF THE ENZYME IS WORKING, IT WILL PRODUCE GLUCOSE.  
IF THE ENZYME IS NOT WORKING, IT WILL NOT PRODUCE GLUCOSE.**

We will see how well the enzyme works after being subjected to different treatments. We test:

- 1) milk + enzyme (our control)
- 2) milk + boiled enzyme (Does boiling stop the enzyme from working?)
- 3) boiled milk + enzyme (Does boiling the substrate stop the enzyme from working?)  
Hint: is the substrate a protein or a sugar?)
- 4) plain, untreated milk (Does milk have glucose without enzyme treatment?)

*All the milk we are testing has had enzyme added to it already, because the enzyme works too slowly for us to add it in class.* To test the various combinations, make a serial dilution of the enzyme-treated milk: pure, 1:10, 1:100. Do this for each of the 4 kinds of combinations. Test each with TesTape. Repeat for 3 trials of each combination.

**SAFETY: TesTape IS FOR EXTERNAL USE ONLY. IT DOES NOT BELONG NEAR YOUR MOUTH. WASH HANDS WITH SOAP AFTER YOU ARE DONE**

TesTape is used by people who need to test the amount of sugar in their urine because they have diabetes. If they find sugar, it means they have too much in their blood and they must do something about it, such as take insulin. Thus, TesTape is a very sensitive test for glucose. Lives depend on it working well.

Most people make lactase enzyme when they are young. As they get older, milk is not as important a part of their diet. Many people no longer make lactase as they get older. They then have trouble digesting dairy products. The lactase enzyme they can no longer produce for themselves can be added to dairy products before they eat them. That is why it was easy to buy lactase for the experiment. Do not try adding lactase to food without following package directions and without parental supervision.

## LACTASE LAB EXTENSION THE EFFECT OF pH ON LACTASE ACTION

Extend your lactase lab to investigate the effects of pH on lactase action. Most enzymes can only do their work within a relatively narrow range of pH values. Should the environment become too acidic or too alkaline (too basic), the enzyme becomes denatured and cannot work. Different enzymes work best under different conditions. One that must work in the acidic environment of the stomach differs in this way from one that operates in the more basic interior of the small intestine.

Using a pH meter or universal indicator paper, make a vinegar/distilled water solution of pH 3. Make a sodium bicarbonate /distilled water solution of pH 10.

Test a control solution. In the bottom right well of your culture plate, put 5 drops of distilled water and 1 drop of lactose solution. Test the pH of the mixture. Add one drop of lactase solution. Wait 5 minutes and test for glucose production with TesTape.

Starting from well A-1, make a serial dilution of the vinegar solution from pure to 1:1000. Add a drop of lactose solution to each well. Test and record the pH of each well. Add a drop of lactase solution to each well. After 5 minutes, test each mixture for glucose production with TesTape.

Repeat the vinegar test for 2 more rows. Did you get similar results each time?

Make a serial dilution of the sodium bicarbonate/distilled water solution from pure to 1:1000. Add a drop of lactose solution to each well. Test and record the pH of each well. Add a drop of lactase solution to each well. After 5 minutes, test each mixture for glucose production with TesTape.

Repeat the sodium bicarbonate solution for 2 more rows.

How could you modify this experiment to make it more precise? more accurate? What other acids and bases could make the experiment more realistic? Could any other chemical interactions modify your results in the changed experimental conditions?