

Investigating Enzymes - Effect of Temperature on Enzyme Activity

Teacher's Guide

This guide is a continuation of the previous laboratory documents. Presented below are notes and comments regarding the laboratory material and preparation for the laboratory exercise.

Important Consideration: This laboratory is structured for five different temperatures. This may be very impractical for many classrooms. If heat blocks or water baths are unavailable for this laboratory, there are several alternative methods to create differential temperatures for incubation. Here are some options:

- Room Temperature – assume this to be around 22°C
- Holding – a fist wrapped around a tube is about 37°C
- Ice Bath – the cafeteria or gym might have flaked ice. Sticking tubes in ice will give a temperature of 0°C
- Hot Tap Water – Right out of the faucet, water should be between 55-60°C. A good size beaker will hold temperature for several minutes.
- Freshly Boiled Water – Take this off the hot plate before using, but it will have a temperature near 100°C

If using such alternative methods of temperature control, focus on the extreme temperatures so a significant difference can be observed. For instance, ice, room temperature and boiled water will give very clear results.

Chemical reactions are influenced by temperature. At low temperatures, reactions slow down because the molecules have less energy. As the temperature increases, the rate of a reaction increases as well. If the temperature is too high, then some of the chemicals in the reaction can actually be destroyed, and the reaction will stop. Enzymes are catalysts to specific biological reactions, and are influenced by temperature just the same.

In this lab, you will measure enzyme activity in a solution and measure the effect of five different temperatures: 30°C, 40°C, 50°C, 60°C, and 70°C. Using a color key, you will be able to determine the speed of the enzyme reaction at these different temperatures. You will also be able to determine the approximate optimal temperature, or the temperature at which enzyme activity is greatest.

Materials

To ensure sufficient quantities, stock solution will be made. Each group should have:

- TRIS, pH 8 (buffer): about 15 drops
- Distilled Water: about 6 drops
- PMS/INT/NAD: about 15 drops
- Lactic Acid (substrate): about 15 drops
- Enzyme (lactate dehydrogenase): about 12 drops

- f. **Stop Reagent**: about 7 drops
- g. 6 reaction tubes
- h. Incubators/Water baths
- i. 6 Droppers/Pipettes
- j. Test Tube Rack

Method

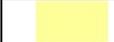
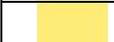
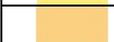
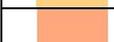
1. You will be running enzyme reactions with five different temperatures. You will run one reaction at each temperature with enzyme and one reaction at 30°C without enzyme (as a negative control) for a total of six reactions. **Teacher's Note: The negative control is important so that degrees of color development can be assessed. All temperatures will give some color development as the simple act of mixing the reagents occurs at room temperature. However, once the reactants equilibrate to the various temperatures, obvious differences should be apparent.**
2. Label the six tubes #1 through #6. As you will be combining different reagents to prepare this reaction, each dropper should be used with only one ingredient. Mixing up the droppers will cause contamination of the ingredients which may cause incorrect results. **TAKE CARE SO THAT YOU DON'T CONTAMINATE THE DIFFERENT TUBES.**
3. Add 2 drops of lactic acid, 2 drops of PMS/INT/NAD, and 2 drops of buffer to the tubes. Into tube #1 add 2 drops of water.
4. It is important that before you start the enzyme reaction that you have the Stop Reagent ready with a pipette. The enzyme reaction is timed and halted by the addition of Stop Reagent, therefore you need to have it ready. **Teacher's Note: The Stop Reagent contains copper sulfate which disrupts the flow of electrons to INT. Students need to add Stop Reagent to the tubes quickly so that tubes don't incubate for different times. You can give the students the option, to add enzyme at 30 second intervals and then add the Stop Reagent at the same intervals so that all tubes incubate equally.**
5. **This is a timed reaction, therefore it is important that when adding the enzyme (which starts the reaction) all tubes start at the same time. Move quickly and accurately when adding enzyme and placing the tubes in the incubators/water baths.** Into tubes #2 through #6, add 2 drops of enzyme. Shake the tubes gently to mix the reagents. Place the reaction tubes into incubators/water baths as follows:

#1	30°C
#2	30°C
#3	40°C
#4	50°C
#5	60°C
#6	70°C
6. Start timing the reaction. After three minutes, examine the tubes. If significant color has developed, continue on to step 7. If little color has developed, wait five minutes and continue to step 7. **Teacher's Note: Color of the tubes should not be dark, but rather differentiating. If clear color differences can be seen between tubes, then the reactions can be stopped.**

7. Add 1 drop of Stop Reagent into each reaction tube to halt the reaction.
8. Score and record the color in each test tube using the color key.
9. Complete the laboratory worksheet once the data has been collected.

Effect of Temperature on Enzyme Activity Worksheet

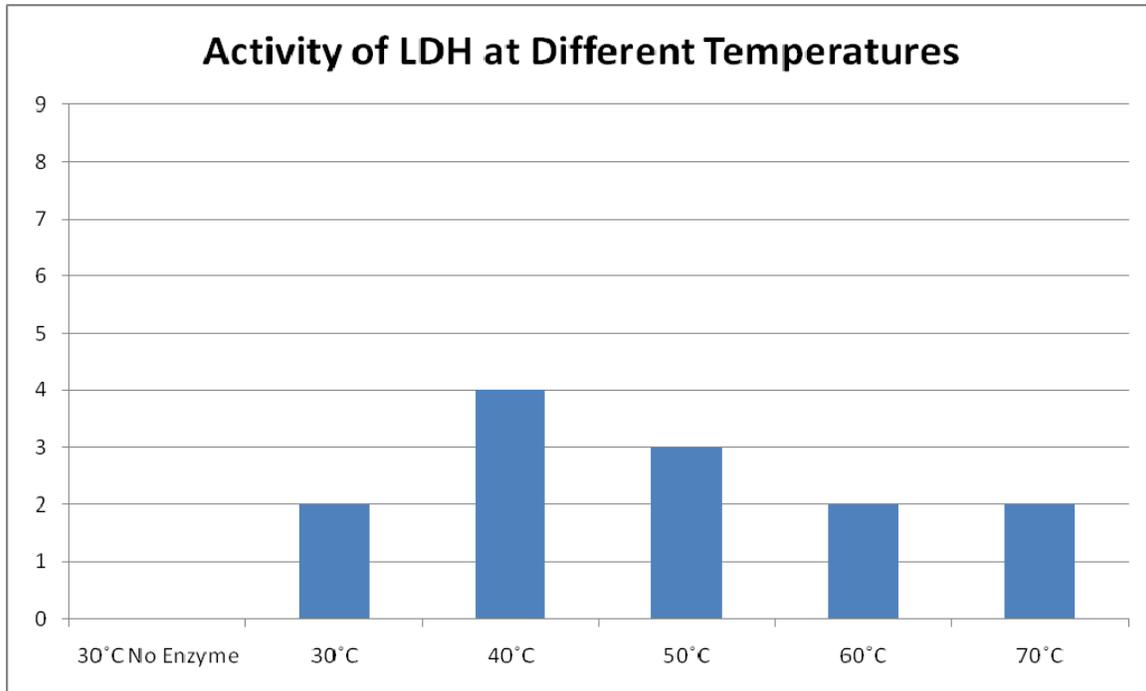
Color Key:

	Color	Score
	Light Yellow	0
	Medium Yellow	1
	Dark Yellow	2
	Light Pink/Purple	3
	Medium Pink/Purple	4
	Dark Pink/Purple	5
	Light Red/Purple	6
	Medium Red/Purple	7
	Dark Red/Purple	8
	Very Dark Red/Purple	9

1. **Visual Optical Density:** Record your observations in the following table.

Temperature	Color Score
Tube 1 - 30°C	0
Tube 2 - 30°C	2
Tube 3 - 40°C	4
Tube 4 - 50°C	3
Tube 5 - 60°C	2
Tube 6 - 70°C	2

2. **Graph Results:** Plot your data below using a bar graph. **Teacher's Note:** This data is representative of what can be expected. In this case, the student stopped the reaction when a distinction between tubes could be made, and not when one tube was very dark.



3. Questions

- a. At which temperature was there the most enzyme activity? How can you tell?
The enzyme was most active at 40°C. Based on the score of the reaction, the plotted data shows the greatest bar height at this temperature.
- b. Why did you add two drops of water instead of enzyme to tube 1?
Tube #1, or the 30°C reaction without enzyme, is a negative control. This reaction should not work and thus remain light yellow. It provides us with a tube that compares no enzyme activity to enzyme activity at the different temperatures. It is possible that the tube without enzyme may change color on its own. Therefore, any reaction that has a different color than the negative control can be attributed to the enzyme at that specific temperature.
- c. What is the importance of knowing the optimal temperature of an enzymatic reaction?
Enzymes can be used outside of cells in many applications such as processing foods, cleaning clothes, and in diagnostics tests. When using enzymes it is important to have them work efficiently by creating an environment that allows high levels of activity. Temperature is one variable that can be changed to allow enzymes to reach their optimal activity.