Simple Enzyme Inquiry Activities for AP Biology

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Vernier Software & Technology
www.vernier.com
888.837.6437

Colleen McDaniel
biology@vernier.com

NSTA National 2019

St. Louis, MO

HANDS-ON ACTIVITIES

Testing Enzyme Activity 2 Ways

- Go Direct Gas Pressure
- Go Direct SpectroVis Plus

PRELIMINARY ACTIVITY FOR

Testing Enzyme Activity

(Gas Pressure Sensor)

Open Inquiry Version

Many organisms can decompose hydrogen peroxide (H_2O_2) enzymatically. Enzymes are globular proteins, responsible for most of the chemical activities of living organisms. They act as *catalysts*, substances that speed up chemical reactions without being destroyed or altered during the process. Enzymes are extremely efficient and may be used over and over again. One enzyme may catalyze thousands of reactions every second.

H_2O_2 is toxic to most living organisms. Many organisms are capable of enzymatically destroying the H_2O_2 before it can do much damage. H_2O_2can be converted to oxygen and water as follows:

$$2\ H_2O_2(aq) \rightarrow 2\ H_2O + O_2(g)$$

Although this reaction occurs spontaneously, enzymes increase the rate considerably. At least two different enzymes are known to catalyze this reaction: *catalase*, found in animals and protists, and *peroxidase*, found in plants. A great deal can be learned about enzymes by studying the rates of enzyme-catalyzed reactions.

In this Preliminary Activity, you will use catalase in yeast to catalytically decompose hydrogen peroxide. You will use a Gas Pressure Sensor to determine the rate of catalase activity by measuring pressure caused by oxygen gas produced as H_2O_2 is decomposed.

Before data collection begins, there is no product, and the pressure is the same as atmospheric pressure. Shortly after data collection begins, oxygen accumulates at a rather constant rate. The slope of the curve at this initial time is constant and is called the initial rate. In this investigation, we will refer to this as the rate of catalase activity. As the peroxide is decomposed, less of it is available to react and the O_2 is produced at lower rates. When no more peroxide is left, O_2 is no longer produced. When data collection is complete, you will perform a linear fit on the resultant graph to determine catalase activity.

After completing the Preliminary Activity, you will use reference sources to find out more about catalase, enzymes, and enzyme activity, and then you will choose and investigate a researchable question dealing with catalase activity. Some topics to consider in your reference search include the following:

- catalyst
- enzyme
- catalase
- hydrogen peroxide
- collision theory
- reaction rate

PROCEDURE

1. Obtain and wear goggles.
2. Start the data-collection program
3. Connect a Gas Pressure Sensor to your Chromebook, computer, or mobile device. Use an interface if necessary.

Figure 1

4. Set up the investigation apparatus.
   a. Measure out 50 mL of 1.5% H_2O_2 into a 125 mL Erlenmeyer flask.
   b. Carefully place a stir bar into the flask.
   c. Use a utility clamp to fasten the flask to the Stir Station as shown in Figure 1.
   d. Position the flask at the center of the Stir Station. Test the stirring speed. **Note**: Select a moderately slow stirring speed that you will use throughout this investigation, including your work on a researchable question, and note the position of the control knob.
   e. Stop the stirrer.
   f. Use the plastic tubing with two Luer-lock connectors to connect the two-hole rubber stopper assembly to the Gas Pressure Sensor, as shown in Figure 1. About one-half turn of the fittings will secure the tubing tightly. The valve connected to the stopper should stay closed during this investigation.
5. Initiate the enzyme catalyzed reaction and start data collection. **Note**: The next steps should be completed as rapidly as possible.
   a. Using a micropipette, add 100 µL of enzyme suspension to the contents of the Erlenmeyer flask.
   b. Tightly seal the flask by twisting in the two-hole stopper connected to the Gas Pressure Sensor.
   c. Ensure that the flask is properly positioned. Turn the stirrer on to the predetermined setting.
   d. Start data collection. **Note**: If the pressure exceeds 130 kPa, the pressure inside the flask will be too great and the rubber stopper is likely to pop off. Carefully remove the stopper from the flask if the pressure exceeds 130 kPa.

6. When 200 seconds have elapsed, stop data collection.

7. Carefully remove the stopper from the flask to relieve the pressure. Dispose of the contents of the flask as directed.

8. Perform a linear fit on the 50–200 s portion of the graph. Record the slope of the line, *m*, as the rate of catalase activity, in kPa/s.

QUESTIONS

1. What rate of catalase activity did you obtain in the Preliminary Activity?

2. Why is it important that cells contain catalase?

3. List three factors that could possibly affect catalase activity.

4. List at least one researchable question concerning catalase activity.

Testing Enzyme Activity

(Gas Pressure Sensor)

OVERVIEW

In the Preliminary Activity, your students will use catalase in yeast to catalytically decompose hydrogen peroxide according to the equation:

$$2\ H_2O_2(aq) \rightarrow 2\ H_2O + O_2(g)$$

They will use a Gas Pressure Sensor to determine the rate of catalase activity by measuring pressure caused by oxygen gas produced as H_2O_2 is decomposed.

During the subsequent Inquiry Process, your students will first find out more about catalase, enzymes, and enzyme activity using the course textbook, other available books, and the Internet. They will then generate and investigate researchable questions dealing with catalase activity. (In the Guided Inquiry approach, students will plan and conduct investigations of the researchable question(s) assigned by you.)

LEARNING OUTCOMES

- Identify variables, design and perform the investigation, collect data, analyze data, draw a conclusion, and formulate a knowledge claim based on evidence from the investigation.
- Measure the production of oxygen gas as hydrogen peroxide is decomposed by the enzyme catalase.
- Gain increased understanding of factors affecting catalase activity.

CORRELATIONS

AP Biology Concept

Essential knowledge 2.D.1: All biological systems from cells and organisms to populations, communities and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.

Essential knowledge 2.D.3: Biological systems are affected by disruptions to their dynamic homeostasis.

Essential knowledge 4.A.1: The subcomponents of biological molecules and their sequence determine the properties of that molecule.

Essential knowledge 4.B.1: Interactions between molecules affect their structure and function.

IB Biology Core Topic and Option

Topic 2.5: Enzymes

Next Generation Science Standards (NGSS)

Science and Engineering Practices	Disciplinary Core Ideas	Crosscutting Concepts
Analyzing and Interpreting Data Developing and Using Models Planning and Carrying Out Investigations	LS1.A: Structure and Function	Cause and Effect Structure and Function

ESTIMATED TIME FOR THE INQUIRY PROCESS

See Appendix C for more information on carrying out each phase of an inquiry experiment.

Inquiry Phase		Open Inquiry	Guided Inquiry
I	Preliminary Activity	30 minutes	30 minutes
II	Generating Researchable Questions (Omitted in Guided Inquiry Approach)	10 minutes	0 minutes
III	Planning	15 minutes	15 minutes
IV	Carrying Out the Plan	40 minutes	40 minutes
V	Organizing the Data	10 minutes	10 minutes
VI	Communicating the Results	15 minutes	10 minutes
VII	Conclusion	5 minutes	5 minutes

MATERIALS

Make the following materials available for student use. Items in bold are needed for the Preliminary Activity.

data-collection interface (if necessary)
data-collection program
Gas Pressure Sensor
Stir Station
50 mL graduated cylinder
distilled water
125 mL Erlenmeyer flask
magnetic stir bar
1.5% H_2O_2 solution
enzyme suspension
utility clamp
tubing with two Luer-lock connectors
two-hole rubber stopper assembly
20–200 µL micropipette*
200 µL micropipette tips
goggles
others as requested by students

*dropper pipettes can be substituted

PRELIMINARY ACTIVITY

Sample Results

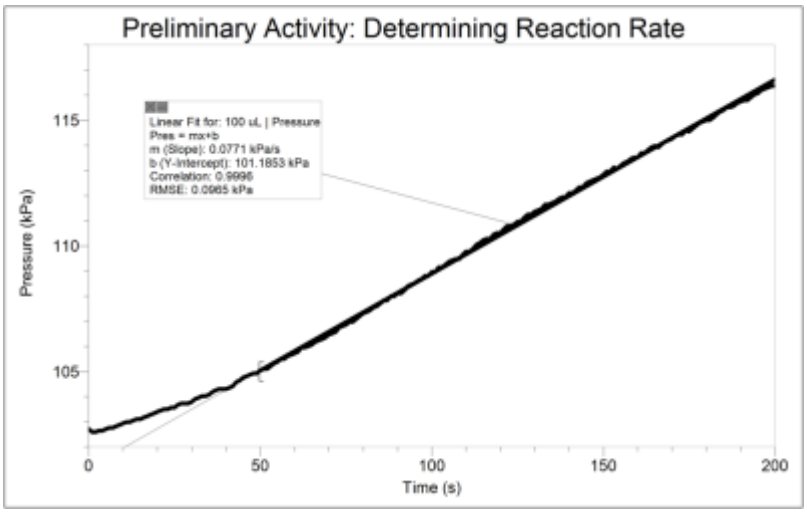

Figure 1 Preliminary Activity Sample Results

Answers to the Questions

1. Answers will vary. The value shown in Figure 1 is 0.0771 kPa/s.

2. Hydrogen peroxide is a harmful by-product of many metabolic processes. To prevent damage, it must be quickly converted to less harmful substances. Cells commonly use catalase to quickly catalyze the decomposition of hydrogen peroxide into less reactive oxygen and water.

3. Answers will vary. Some factors affecting catalase activity are temperature, pH, enzyme concentration, enzyme source, substrate used, substrate concentration, the presence of an inhibitor, inhibitor concentration, and the catalyst used.

4. Answers will vary. See the Sample Results and Other Researchable Questions sections for possible answers.

SAMPLE RESULTS

Student results will vary depending on experimental design.

How does temperature affect catalase activity?

Table 1: Temperature Effect

Temperature (ºC)	Mean rate (kPa/s)
5	0.0586
15	0.0805
25	0.1003
35	0.1469
45	0.1202
55	0.1026

Multiple trials at water bath temperatures of 5, 15, 25, 35, 45, and 55ºC were used. Typically, a maximum rate of catalase activity is obtained at a temperature near 35ºC. See the Tips section for water bath setup suggestions.

How does pH affect catalase activity?

Table 2: The Effect of pH	
pH	Mean rate (kPa/s)
4	0.0778
7	0.1118
10	0.1033

The procedure outlined in the student handout was modified so that the 1.5% H_2O_2 was prepared by mixing equal volumes of 3% H_2O_2 and the appropriate buffer. For the pH values tested, catalase activity was lowest at pH 4 and highest at pH 7.

What is the optimal pH for the catalase catalyzed decomposition of hydrogen peroxide? (Advanced)

Table 3: A Closer Look at the Effect of pH	
pH	Catalase activity rate (kPa/s)
5.1	0.0654
6.1	0.0826
7.2	0.0859
8.1	0.0739
8.7	0.0726

This investigation is recommended for advanced students. The 1.5% H_2O_2 solution used at each pH value was prepared by mixing equal volumes of 3% H_2O_2 and the appropriate phosphate buffer. The phosphate buffer solutions used were prepared using combinations of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$) and dibasic sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$) solutions. Such investigations using household baker's yeast generally indicate that catalase activity is high over the range pH 6–8.

How does enzyme concentration affect catalase activity?

Table 3: Effect of H_2O_2 Concentration	
Volume of yeast suspension (µL)	Catalase activity (kPa/s)
50	0.0460
100	0.0770
150	0.1161
200	0.1578

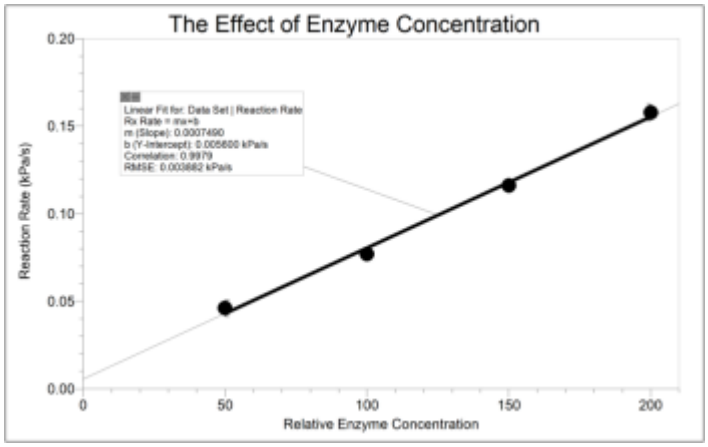

Figure 2 The relationship between reaction rate and yeast concentration

Data were collected at 22ºC using the procedure outlined in the Preliminary Activity and varying the enzyme concentration as described in Table 3. Under the conditions specified, reaction rate was found to vary directly with enzyme concentration, as evidenced by the linear fit shown in Figure 2 with a correlation coefficient of 0.9979 and with the line passing near the origin.

How does hydrogen peroxide concentration affect catalase activity?

Table 4: Effect of H_2O_2 Concentration

Volume 3% H_2O_2 (mL)	Volume water (mL)	Percent H_2O_2 (%)	H_2O_2 concentration (mol/L)	Catalase activity (kPa/s)
1.0	49.0	0.06	0.018	0.0018
5.0	45.0	0.3	0.088	0.0274
10.0	40.0	0.6	0.176	0.0436
20.0	30.0	1.2	0.352	0.0802
30.0	20.0	1.8	0.528	0.0843
40.0	10.0	2.4	0.704	0.0864
50.0	0.0	3.0	0.880	0.0695

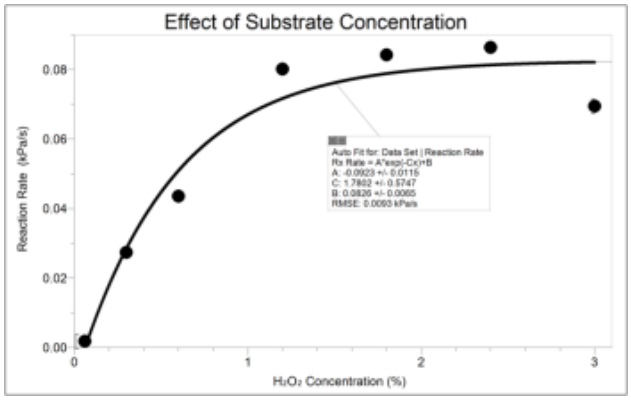

Figure 3 The effect of substrate concentration expressed in percent

Data were collected at 22ºC using the procedure outlined in the Preliminary Activity while varying H_2O_2 concentration as described in Table 4. As can be seen in Figure 3, a natural exponent curve fit matches the data well. The rate of the reaction initially increased rapidly, but then approached a constant rate as the substrate concentration increased.

OTHER RESEARCHABLE QUESTIONS

Recommended for Open Inquiry or Guided Inquiry

- What is the optimal temperature for the catalase catalyzed decomposition of hydrogen peroxide?
- What is the optimal pH for the catalase catalyzed decomposition of hydrogen peroxide?
- How does boiling the catalase affect catalase activity?
- How effectively does ethanol (methanol, ascorbate, formate) inhibit the catalase catalyzed decomposition of hydrogen peroxide?

Recommended for Advanced Students

- What is the effect of catalase type on the rate of enzyme activity?
- What is the effect of organism type (plant, animal, and protist) on the rate of enzyme activity?
- What are the K_{max}, ½ K_{max}, and K_m values for an investigation of the effect of substrate concentration on reaction rate?

There are many more possible researchable questions. Students should choose a researchable question that addresses the learning outcomes of your specific standards. Be sure to emphasize experimental control and variables.

Instructors using the Guided Inquiry approach select the researchable questions to be investigated by their students. We encourage you to assign multiple researchable questions because this strategy enhances student interaction and learning.

TIPS

1. In the Electronic Resources you will find guided- and open-inquiry versions of each student experiment for each supported data-collection software (e.g., Logger *Pro* or Graphical Analysis 4). Sign in to your account at **www.vernier.com/account** to access the Electronic Resources. See Appendix A for more information. **Note**: The printed version of the book and the PDF of the entire book (found in the Electronic Resources) include only the Open Inquiry versions of the experiments.

2. Test the experiment before the students begin to determine enzyme activity. Depending on the type of enzyme you use, the activity will vary greatly, you may need to dilute the enzyme solution or make a new solution to get the ideal reaction rate.

3. High concentration combinations that produce high pressure increase rates should be avoided in this investigation. Higher concentrations can cause significant temperature increases and greater apparent deviation from expected results. Stoppers tend to pop out of 125 mL

Erlenmeyer flasks at pressures above 130 kPa, and greater concentration combinations tend to exacerbate this problem.

4. If micropipets are not available, dropper pipettes can be substituted with number of drops of enzyme suspension delivered being counted.

5. Many different organisms may be used as a source of catalase in this experiment including beef liver, potato, or living yeast.

6. We recommend purchasing purified catalase enzyme from Flinn Scientific, Ward's Natural Science, or Sigma-Aldrich. The concentration of enzyme varies from 2000–5000 units/mg and depends on the bottle. Store the catalase powder as instructed. Enzyme activity may decrease from year to year, but will remain viable for up to three years.

7. Use the following instructions to prepare an enzyme solution:

   a. Purified catalase

      i. Make a stock solution of 1000 units/mL.
      ii. Dilute the stock solution to 200 units/mL for use by the students.

   b. Yeast suspension

      i. Dissolve 1 package (7 g) of dried yeast per 100 mL of 2% sugar solution. To prepare a 2% sugar solution, add 20 grams of sugar to make one liter of solution.
      ii. Incubate the suspension in 37–40°C water for at least 10 minutes to activate the yeast.
      iii. To ensure a uniform yeast concentration, make the suspension available on magnetic stirrer and instruct your students to withdraw their samples from the center as the suspension is being stirred.
      iv. The yeast may need to be diluted if the reaction occurs too rapidly.

   c. Liver suspension

      i. Homogenize 0.5 to 1.5 g of beef liver in 100 mL of cold water.
      ii. Keep the suspension on ice until it is to be used.
      iii. Dilute the suspension as needed based on reaction rate.

8. The 3% H_2O_2 (GHS Signal Word: **DANGER**) may be purchased from any supermarket. The 1.5% H_2O_2 used in the Preliminary Activity can be prepared by mixing equal volumes of 3% H_2O_2 and distilled water. If the H_2O_2 solution is refrigerated, bring it to room temperature before use.

9. Vernier Software & Technology sells a pH buffer package for preparing buffer solutions with pH values of 4, 7, and 10 (order code: PH-BUFCAP). Simply add the capsule contents to 100 mL of distilled water.

10. You can also prepare pH 4, 7, and 10 buffers using the following recipes:

    - pH 4: Add 2.0 mL of 0.1 M HCl (GHS Signal Word: **DANGER**) to 1000 mL of 0.1 M potassium hydrogen phthalate.

- pH 7: Add 582 mL of 0.1 M NaOH (GHS Signal Word: **WARNING**) to 1000 mL of 0.1 M potassium dihydrogen phosphate.
- pH 10: Add 214 mL of 0.1 M NaOH (GHS Signal Word: **WARNING**) to 1000 mL of 0.05 M sodium bicarbonate.

Store the solutions in capped containers at 4°C. Buffer solutions are stable for weeks at this temperature.

11. Prepare 0.1 M sodium phosphate buffer stock solutions, used in the closer look at the effect of pH on catalase activity, as follows:

    - Monobasic sodium phosphate: Add 13.80 g of $NaH_2PO_4 \cdot H_2O$ to distilled water to make a total of 1 L of solution.
    - Dibasic sodium phosphate: Add 26.81 mL of $Na_2HPO_4 \cdot 7H_2O$ to distilled water to make a total of 1 L of solution.

    Store the solutions in capped containers at 4°C. Buffer solutions are stable for weeks at this temperature.

    Mix monobasic and dibasic sodium phosphate buffer solutions to prepare sodium phosphate buffer solutions with various desired pH values in the pH 4.8–9.0 range. Store the solutions in capped containers at 4°C. Such buffer solutions are stable for weeks at this temperature.

12. The two-hole stopper used in this investigation is one of the stoppers that comes with the Gas Pressure Sensor. The stopper is fitted with two Luer lock connections. Your students will use the clear tubing, which also comes with the Gas Pressure Sensor, to connect the two-hole stopper to the sensor. Remind your students that a 1/2 to 3/4 turn of the Luer lock is sufficient to tighten the connection. Tightening down the Luer lock too much can damage the fittings. The valve connected to the second Luer lock connection stays closed during this investigation.

13. A magnetic stirrer is used in this investigation to ensure uniform mixing, and, more importantly, to expel oxygen from the liquid phase. A Vernier Stir Station set at its middle stir speed, 5, works very well.

14. The water added to the reaction mixtures serves as a heat sink for the very exothermic reaction. It also serves to reduce the volume of the gas phase in the flask, and thus increase pressure readings.

15. A water bath is needed in investigations of the effect of temperature on reaction rate. A water bath, similar to the one shown in Figure 4, can be made using a 1 gallon plastic bottle. Cut the bottle off at a height of 9 cm. Common beakers do not work because they do not simultaneously provide sufficient volume and allow the utility clamp-held flask to reach the bottom.

Figure 4

16. Emphasize to your students the importance of providing an airtight fit with all plastic-tubing connections and when twisting the stopper into a flask.

17. We recommend an excellent article entitled "A Quick and Accurate Oxygen-Based Pressure-Sensor Assay for Catalase Activity" found in the October, 2009 edition of the *Journal of Chemical Education* (Megan E. Lewis, Rebecca M. Levine, John T. York, and W. Tandy Grubbs, vol. 86, no. 10, pp. 1227–1230). The article discusses the chemistry of the catalase catalyzed decomposition of hydrogen peroxide, procedure alternatives, and sample results, and it provides much other helpful information for instructors using this inquiry investigation at the high school and introductory college levels.

18. For additional information about the Vernier probeware used in this experiment, including tips and product specifications, visit **www.vernier.com/manuals** and download the appropriate user manual.

19. The experimental plans that your students submit for approval should list laboratory safety concerns, including chemical safety concerns, and specify how they will address these safety concerns during their investigations.

HAZARD ALERTS

The chemical safety signal words used in this experiment (**DANGER** and **WARNING**) are part of the Globally Harmonized System of Classification and labeling of Chemicals (GHS). Refer to the Safety Data Sheet (SDS) that came with the chemical for proper handling, storage, and disposal information. These can also be found online from the manufacturer. See Appendix F for more information.

Hydrogen peroxide, 3%: **DANGER**: Causes skin and eye irritation.

Hydrochloric acid, 0.1 M, HCl: **DANGER**: Causes severe skin and eye damage. Do not breathe mist, vapors, or spray. May cause respiratory irritation. May be harmful if swallowed. Industrial exposure to vapors and mists is listed as a known human carcinogen by International Agency for Research on Cancer (IARC).

Sodium hydroxide, 0.1 M, NaOH: **WARNING**: Causes skin and eye irritation.

PRELIMINARY ACTIVITY FOR

Testing Enzyme Activity

(Spectrometer)

Open Inquiry Version

Enzymes are molecules that regulate the chemical reactions that occur sin all living organisms. Almost all enzymes are globular proteins that act as *catalysts*, substances that speed up chemical reactions. Enzymes catalyze reactions by reducing the activation energy for a specific reaction to occur and yet are neither destroyed nor altered during this process. At the molecular level, enzymes catalyze these reactions by briefly binding to the substrate or reactants to form an enzyme-substrate complex. The reaction takes place while the substrate is bound to the enzyme, converting the substrate to the new product. The new product is then released from the enzyme substrate complex and the enzyme is then free to bind with more substrate.

$$\text{Enzyme} + \text{Substrate} \rightarrow \text{Enzyme-Substrate Complex} \rightarrow \text{Enzyme} + \text{Product}$$

H_2O_2 is toxic to most living organisms. Many organisms are capable of enzymatically destroying the H_2O_2 before it can do much damage. H_2O_2can be converted to oxygen and water as follows:

$$2\ H_2O_2(aq) \rightarrow 2\ H_2O + O_2(g)$$

Although this reaction occurs spontaneously, enzymes increase the rate considerably. At least two different enzymes are known to catalyze this reaction: *catalase*, found in animals and protists, and *peroxidase*, found in plants. A great deal can be learned about enzymes by studying the rates of enzyme-catalyzed reactions.

In this Preliminary Activity, you will use a colorimetric assay to determine the rate of reaction of the enzyme peroxidase.

After completing the Preliminary Activity, you will use reference sources to find out more about enzymes, turnips, and peroxidase, and then you will choose and investigate a researchable question. Some topics to consider in your reference search include the following:

- catalyst
- enzyme
- substrate
- peroxidase
- proteins
- denature
- Michaelis-Menten constant
- Lineweaver-Burk plot

PROCEDURE

1. Obtain and wear goggles.

2. Obtain three test tubes. Label one test tube with the letter **B**, another with the letter **E**, and the last with the letter **S**.

3. Prepare a blank solution by filling the test tube labeled B with 2 mL of pH 5 reaction buffer, 1 mL of 0.02% hydrogen peroxide, 0.5 mL of 0.2% guaiacol, and 1 mL of extraction buffer. **DANGER**: *Guaiacol solution, 0.2%: Highly flammable liquid and vapor. Keep away from heat, sparks, open flames, and hot surfaces. May be harmful if swallowed. Causes skin and eye irritation. May cause drowsiness or dizziness. Avoid breathing mist, vapors or spray.*

4. Transfer 3 mL of the blank solution to a cuvette using a pipette. To correctly use cuvettes, remember:

   - Wipe the outside of each cuvette with a lint-free tissue.
   - Handle cuvettes only by the top edge of the ribbed sides.
   - Dislodge any bubbles by gently tapping the cuvette on a hard surface.
   - Always position the cuvette so the light passes through the clear sides.

5. Launch Spectral Analysis. Connect the SpectroVis Plus to your Chromebook or computer. Click or tap Absorbance vs. Time.

6. To calibrate the spectrophotometer, place the blank cuvette in the spectrophotometer and select Finish Calibration. **Note**: If necessary, wait for the spectrophotometer to warm up before selecting Finish Calibration.

7. To select the appropriate wavelength, enter **500** as the Wavelength value. Click or tap Done.

8. Prepare an enzyme solution by filling the test tube labeled E with 2 mL of pH 5 reaction buffer, 1 mL of enzyme extract, and 1 mL of extraction buffer.

9. Prepare a substrate solution by filling the test tube labeled S with 1 mL of pH 5 reaction buffer, 2 mL of 0.02% hydrogen peroxide, and 1 mL of 0.2% guaiacol.

10. Collect data. Do the following as quickly as possible.

    a. Add the substrate solution to the enzyme solution.
    b. Transfer 3 mL of the combined solution to a cuvette using a pipette.
    c. Cap the cuvette and place it in the spectrophotometer.
    d. Start data collection.
    e. After 200s, click or tap Stop.

11. When data collection is complete, select the initial linear region of your data on the graph. This should correspond to the first 100 seconds of data.

12. Perform a linear fit on the selected portion of the graph. Record the slope of the line, *m*, as the rate of reaction, in absorbance/s.

13. (Optional) Print or save your data.
14. Discard the cuvette contents and other solutions as directed by your instructor.

QUESTIONS

1. What was the rate of reaction?
2. List three factors that could possibly affect the rate of peroxidase catalyzed reactions.
3. List at least one researchable question concerning the peroxidase catalyzed reactions.

Testing Enzyme Activity

(Spectrometer)

OVERVIEW

This investigation is designed to introduce your students to the quantitative analysis of enzymes, using an enzyme that breaks down hydrogen peroxide. Hydrogen peroxide is toxic to most living organisms. Many organisms have enzymes that are capable of enzymatically destroying H_2O_2 before it can do much cellular damage. H_2O_2 can be converted to oxygen and water, as follows:

$$2\ H_2O_2(aq) \rightarrow 2\ H_2O + O_2(g)$$

At least two different enzymes are known to catalyze this reaction: *catalase*, found in animals and protists, and *peroxidase*, which is found in plants. Turnip peroxidase is the enzyme used in this investigation.

In the Preliminary Activity, your students will use a colorimetric assay to determine the rate of reaction of this enzyme. For more thorough background information on peroxidase and the colorimetric assay, see the Background section on pages 1–2 of the Instruction Manual accompanying the Peroxidase Enzyme Activity – Advanced Inquiry Laboratory Kit, Big Idea 4, Investigation 13 (available from Flinn Scientific, Inc., #FB2039)

During the subsequent Inquiry Process, your students will first find out more about enzymes and peroxidase using the course textbook, other available books, and the Internet. They will then generate and investigate researchable questions. (In the Guided Inquiry approach, students will plan and conduct investigations of the researchable question(s) assigned by you.)

LEARNING OUTCOMES

- Identify variables, design and perform the investigation, collect data, analyze data, draw a conclusion, and formulate a knowledge claim based on evidence from the investigation.
- Determine the rate of the peroxidase catalyzed reaction converting H_2O_2 to H_2O and O_2.
- Gain increased understanding of factors affecting peroxidase activity.

CORRELATIONS

AP Biology Concept

Essential knowledge 2.D.1: All biological systems from cells and organisms to populations, communities and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.

Essential knowledge 2.D.3: Biological systems are affected by disruptions to their dynamic homeostasis.

Essential knowledge 4.A.1: The subcomponents of biological molecules and their sequence determine the properties of that molecule.

Essential knowledge 4.B.1: Interactions between molecules affect their structure and function.

IB Biology Core Topic and Option

Topic 2.5: Enzymes

Next Generation Science Standards (NGSS)

Science and Engineering Practices	Disciplinary Core Ideas	Crosscutting Concepts
Analyzing and Interpreting Data Developing and Using Models Planning and Carrying Out Investigations	LS1.A: Structure and Function	Cause and Effect Structure and Function

ESTIMATED TIME FOR THE INQUIRY PROCESS

See Appendix C for more information on carrying out each phase of an inquiry experiment.

Inquiry Phase		Open Inquiry	Guided Inquiry
I	Preliminary Activity	20 minutes	20 minutes
II	Generating Researchable Questions (Omitted in Guided Inquiry Approach)	10 minutes	0 minutes
III	Planning	10 minutes	10 minutes
IV	Carrying Out the Plan	50 minutes	50 minutes
V	Organizing the Data	10 minutes	10 minutes
VI	Communicating the Results	10 minutes	10 minutes
VII	Conclusion	5 minutes	5 minutes

MATERIALS

Make the following materials available for student use. Items in bold are needed for the Preliminary Activity. Many of these materials come in the Peroxidase Enzyme Activity – Advanced Inquiry Laboratory Kit, Big Idea 4, Investigation 13, from Flinn Scientific, Inc. (**www.flinnsci.com**, #FB2039).

data-collection program
spectrophotometer*
three test tubes, 13 × 100 mm**
six 2 mL graduated transfer pipettes***
pipet bulb
two plastic cuvettes with lids
goggles
lint-free tissue
extraction buffer
reaction buffer, pH 5
0.02% H_2O_2
0.2% guaiacol
enzyme extract
test tube rack
others as suggested by students

*Any Vernier-compatible spectrophotometers can be used (e.g., Go Direct SpectroVis Plus or Vernier Spectrometer)
**15 mL conical tubes can be substituted
***Appropriate graduated transfer pipettes (1 and 5 mL) may be substituted

PRELIMINARY ACTIVITY

Sample Results

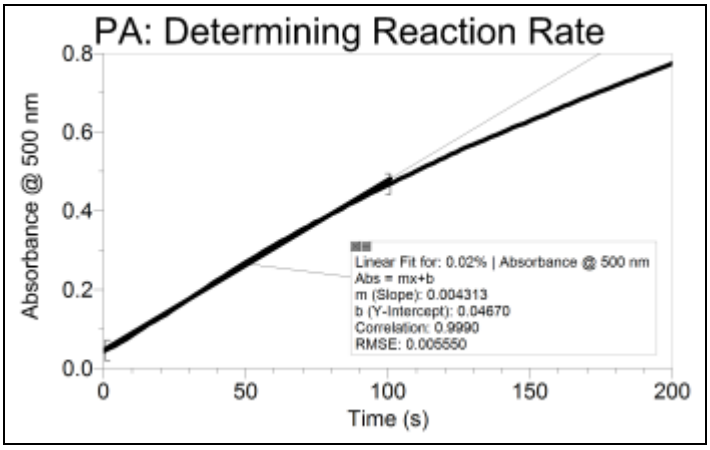

Figure 1 Reaction rate (initial velocity) of the peroxidase catalyzed reaction

Answers to the Questions

1. Answers will vary. The reaction rate determined in Figure 1 is 0.004313 absorbance/s.

2. Some factors affecting the rate of reaction are: enzyme concentration, substrate concentration, source of the peroxidase, temperature, pH, presence of an inhibitor, inhibitor concentration.

3. Answers will vary. See the Sample Results and Other Researchable Questions sections for possible answers.

SAMPLE RESULTS

Student results will vary depending on experimental design.

How does exposing the enzyme extract to various temperatures affect reaction rate?

Table 1: Effect of Temperature	
Exposure Temperature (°C)	Reaction rate (abs/s)
21	0.003633
40	0.003481
60	0.003363
75	0.001139
90	0.0003481
100	0.00007752

These data were collected using a modification of the Preliminary Activity procedure. See the Tips section for details.

The enzyme appears to begin to denature at higher temperatures since the rate of reaction decreases significantly after exposure to higher temperatures.

How does pH affect reaction rate?

Table 2: Effect of pH	
pH	Reaction rate (abs/s)
3	0.0073
4	0.00699
5	0.00646
6	0.00589
8	0.00467
10	0.000398

These data were collected using a modification of the Preliminary Activity procedure. See the Tips section for details.

The rate of reaction decreased with increasing pH over the range of pH values tested. The reaction appears to significantly decrease at pH values higher than 6.

How does enzyme concentration affect reaction rate?

Table 3: Effect of Enzyme Concentration		
Enzyme volume (mL)	Enzyme concentration (Relative)	Reaction rate (abs/s)
2.0	4	0.0044
1.5	3	0.0034
1.0	2	0.0023
0.5	1	0.0011
0.0	0	0.0001

The concentration of the H_2O_2 was held constant at 0.02%. The reaction rate was found to be proportional to enzyme concentration. See the Tips section for more details.

How does substrate concentration affect rates of peroxidase catalyzed reactions?

Table 4: Effect of Substrate Concentration on Reaction Rate	
Substrate concentration (%)	Reaction rate (abs/s)
0.06	0.00488
0.03	0.00458
0.02	0.00412
0.01	0.00312
0.005	0.00202

Table 5: Summary of Estimated Values		
V_{max}	½ V_{max}	K_m
0.00487 abs/s	0.00243 abs/s	0.0066%

Data were collected using the H_2O_2 concentrations specified in Table 4. The rate of the reaction initially increased rapidly but then approached a constant rate as the substrate concentration increased.

The value of the maximum velocity, or V_{max}, was estimated to be 0.00487 abs/s. Accordingly, the estimated value for ½V_{max}, half the maximum velocity, is 0.00243 abs/s, and K_m is then found to be 0.0066% H_2O_2. See the Tips section for more details.

Lineweaver-Burk Plot Using Substrate Concentrations (Advanced)

Table 6: Lineweaver-Burk Plot Data			
Substrate concentration (%)	Reaction rate (abs/s)	Inverse of substrate concentration (1/%)	Inverse of reaction rate (s/abs)
0.06	0.00488	16.67	204.9
0.03	0.00458	33.33	218.3
0.02	0.00412	50.00	242.7
0.01	0.00312	100.00	320.5
0.005	0.00202	200.00	495.1

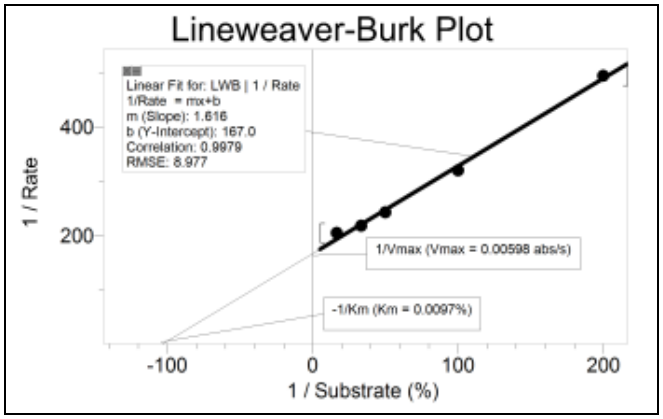

Figure 2 Use of a Lineweaver-Burk plot to determine the apparent V_{max}, and K_m of Turnip Peroxidase

A Lineweaver–Burke plot is much more accurate for calculating the apparent K_m, V_{max}, and ½V_{max} of enzymes. This form of plot makes a plot of reaction rate *vs.* substrate concentration linear. As shown in Figure 2, the y-intercept of the line is equivalent to $1/V_{max}$ and the x-intercept is equivalent to $-1/K_m$. As shown below, the y-intercept, x-intercept, and slope of the line can be used to calculate the Km, V_{max}, and ½V_{max} of peroxidase. Estimated and calculated values for peroxidase using the data presented above are listed in Table 7.

The slope is 1.616, and the y-intercept is 167.0.

y-intercept = $1/V_{max}$ = 167; and V_{max}= 0.00598 abs/s

½V_{max}= ½ (0.00598) = 0.003 abs/s

$X_i = -b/m = -167/1.616 = -103.34$. $1/-103.34 = -0.00967$ and $K_m = -1\ (-0.00967) = 0.00967\%$

Table 7: Comparing Values

	V_{max}	½ V_{max}	K_m
Estimated value	0.00487 abs/s	0.00243 abs/s	0.0066%
Calculated value	0.00599 abs/s	0.00299 abs/s	0.00967%

OTHER RESEARCHABLE QUESTIONS

Recommended for Open Inquiry or Guided Inquiry

- What is the optimal temperature for the peroxidase catalyzed decomposition of hydrogen peroxide?
- How does boiling the enzyme affect peroxidase activity?
- How effectively does ethanol (methanol, ascorbate, formate) inhibit the peroxidase catalyzed decomposition of hydrogen peroxide?

Recommended for Advanced Students

- What is the effect of catalase/peroxidase type on the rate of enzyme activity?
- What is the effect of organism type (plant, animal, and protist) on the rate of enzyme activity?

There are many more possible researchable questions. Students should choose a researchable question that addresses the learning outcomes of your specific standards. Be sure to emphasize experimental control and variables.

Instructors using the Guided Inquiry approach select the researchable questions to be investigated by their students. We encourage you to assign multiple researchable questions because this strategy enhances student interaction and learning.

TIPS

1. In the Electronic Resources you will find guided- and open-inquiry versions of each student experiment for each supported data-collection software (e.g., Logger *Pro* or Graphical Analysis 4). Sign in to your account at **www.vernier.com/account** to access the Electronic Resources. See Appendix A for more information. **Note**: The printed version of the book and the PDF of the entire book (found in the Electronic Resources) include only the Open Inquiry versions of the experiments.

2. The instructions provided assume that you have purchased the Peroxidase Enzyme Activity – Advanced Inquiry Laboratory Kit, Big Idea 4, Investigation 13, from Flinn Scientific, Inc. (**www.flinnsci.com**, #FB2039). However, hydrogen peroxide can be purchased from most stores and instructions on making buffers from salts are also provided.

3. The reaction being studied in this investigation is temperature sensitive. **Important**: Be sure to bring all reagents to room temperature before use.

4. Each student group will require the following solutions in the specified amounts for the Preliminary Activity:

    - 6 mL of reaction buffer, pH 5
    - 4 mL of 0.02% H_2O_2
    - 3 mL of 0.2% guaiacol (GHS Signal Word: **DANGER**)
    - 3 mL of extraction buffer
    - 2 mL of enzyme extract (diluted from stock solution)

5. To prepare the extraction buffer:
    a. Mix 250 mL of 0.2 M sodium phosphate monobasic solution with 250 mL of 0.2 M sodium phosphate dibasic solution.
    b. Bring solution above to 1 L with distilled or deionized water. The resulting solution is a pH 7, 0.1 M sodium phosphate buffer that is referred to as the extraction buffer.
    c. Store in a capped container at 4ºC. This solution is stable for weeks at this temperature.
    d. The solution should be at room temperature when used.

6. To prepare the reaction buffer, pH 5:
   a. Dissolve the pH 5 buffer envelope in 500 mL of distilled or deionized water according to packet instructions.
   b. Store in a capped container at 4ºC. This pH buffer can be stored for weeks at this temperature. The solution should be at room temperature when used.
7. To prepare 0.02% hydrogen peroxide:
   a. Dilute 3 mL of 3% hydrogen peroxide (GHS Signal Word: **DANGER**) to a final volume of 500 mL using distilled or deionized water.
   b. Store in a dark capped container at 4ºC. This hydrogen peroxide solution can be stored for a week at this temperature.
   c. The solution should be at room temperature when used.
8. To prepare the stock solution of enzyme extract:
   a. Peel and cut a turnip root and cut into small pieces.
   b. Place 6 g of turnip in a blender. Add 500 mL of ice-cold extraction buffer.
   c. Blend for 6 minutes using 1 minute bursts. Wait 2 minutes between bursts.
   d. Filter through filter paper into a pre-chilled container on ice.
   e. Store in a dark capped container at 4ºC. The enzyme extract can be stored for a week at this temperature.
   f. The solution should be at room temperature when used.
9. Many spectrophotometers, including the SpectroVis Plus, begin to lose linearity above an absorbance of 1.0. For this reason, you will need to determine the enzyme activity of the stock solution of enzyme extract. You will then need to dilute the stock solution to make the student enzyme extract:
   a. Add 1 mL of stock enzyme extract to 9 mL of extraction buffer.
   b. Test the resulting solution by following the Preliminary Activity instructions.
   c. In order to get good data, the absorbance at the end of the experiment should not be greater than 1.
   d. If the ending absorbance is greater than 1, the enzyme activity is too high. Repeat the test after diluting the enzyme extract further.
   e. Repeat until the appropriate relationship is observed. Good data will be obtained if the final absorbance reading (after 200 seconds) is between 0.8–1.
   f. Prepare enough enzyme extract for your students. Dilute the stock enzyme extract solution as you determined above to make the student enzyme extract. Use extraction buffer for the dilution.
   g. Each student will need 2 mL of enzyme extract to complete the Preliminary Activity.
10. Make sure that only the initial rate is used when calculating the slope of each run. This typically corresponds with the first 100 seconds of data collected.

11. Tips for determining the effect of temperature exposure on enzyme reaction rate:

    - The enzyme extract can be pipeted into test tubes or 1.5 mL microcentrifuge tubes that are then placed in water baths at the desired temperatures.
    - The enzyme extract should be exposed to the specified temperature for at least 5 minutes.
    - The enzyme extract should be returned to room temperature before determining the reaction rate.

12. Tips for determining the effect of pH on enzyme reaction rate:

    - pH buffer envelopes are provided in the Peroxidase Enzyme Activity – Advanced Inquiry Laboratory Kit, Big Idea 4, Investigation 13, from Flinn Scientific, Inc. (**www.flinnsci.com**, Catalog # FB2039)
    - Dissolve the desired pH buffer envelope in 500 mL of distilled or deionized water according to packet instructions. Prepare separate buffer solutions for each desired pH.
    - Using the instructions in the Preliminary Activity, substitute the desired pH buffer for the pH 5, reaction buffer.

13. To create your own series of pH phosphate buffers, prepare 0.1 M sodium phosphate buffer stock solutions as follows:

    - Monobasic sodium phosphate: Add 13.80 g of $NaH_2PO_4 \cdot H_2O$ to distilled water to make a total of 1 L of solution.
    - Dibasic sodium phosphate: Add 26.81 mL of $Na_2HPO_4 \cdot 7H_2O$ to distilled water to make a total of 1 L of solution.
    - Store the solutions in capped containers at 4°C. Buffer solutions are stable for weeks at this temperature.
    - Mix monobasic and dibasic sodium phosphate buffer solutions to prepare sodium phosphate buffer solutions with various desired pH values in the pH 4.8–9.0 range. Store the solutions in capped containers at 4°C. Such buffer solutions are stable for weeks at this temperature.

14. Tips for the determining the effect of peroxidase concentration on reaction rate:

    - The enzyme solution in the Preliminary Activity is composed of 2 mL of pH 5 reaction buffer, 1 mL of enzyme extract, and 1 mL of extraction buffer.
    - Your students can create different concentrations of enzyme by always using 2 mL of pH 5 reaction buffer and 2 mL of solution containing both enzyme extract and extraction buffer.
    - Workable combinations of enzyme extract, extraction buffer, and reaction buffer are presented in Table 8.

Table 8: A Possible Series of Enzyme Concentrations				
Test Tube	Enzyme extract (mL)	Extraction buffer (mL)	Reaction buffer (mL)	Final volume (mL)
1	2.0	0.0	2	4
2	1.5	0.5	2	4
3	1.0	1.0	2	4
4	0.5	1.5	2	4
5	0	2	2	4

15. Tips for the determining the effect of substrate concentration on reaction rate:
    - The substrate solution in the Preliminary Activity is composed of 1 mL of pH 5 reaction buffer, 2 mL of 0.02% H_2O_2, and 1 mL of 0.2% guaiacol. Your students can create different substrate concentrations by always using 1 mL of pH 5 reaction buffer and 1 mL of 0.2% guaiacol, and then using 2 mL of different concentrations of H_2O_2.
    - Results will vary, but good V_{max} values were found using 0.06% H_2O_2. To create a stock solution of 0.06% H_2O_2, add 2 mL of 3% $H_2O_2$to 98 mL of deionized or distilled water.
    - Use the stock solution of 0.06% H_2O_2, to create substrate concentrations at 0.03, 0.02, 0.01, and 0.005%.
16. Estimated V_{max}, $\frac{1}{2}V_{max}$, and K_m values can be obtained using Logger *Pro* or LabQuest App following these steps:
    a. Extend the graph of rate *vs.* substrate concentration to 0.08%.
    b. Produce a natural exponent curve fit.
    c. Use the Interpolate feature to obtain an estimated value for V_{max}.
    d. Divide the V_{max} value by 2 to obtain $\frac{1}{2}V_{max}$.
    e. To obtain an estimated value for K_m, use the Interpolate feature to obtain the H_2O_2 concentration corresponding to the estimated value of $\frac{1}{2}V_{max}$.
17. Commercial hydrogen peroxide, which can be purchased at any supermarket, is used for this investigation. If it is refrigerated, bring it to room temperature before use. It is sold as a 3% H_2O_2 solution by weight. Assuming a 3.0% concentration and a density of 1.00 g/mL, the concentration of this H_2O_2 is 0.88 M. The molar concentration of diluted H_2O_2 solutions can be calculated using this formula:

    $$[H_2O_2] = 0.88\ M \times (\%\ H_2O_2/3\%\ H_2O_2)$$

    For example, 2% H_2O_2= 0.88 M × (2/3) = 0.59 M H_2O_2

18. For additional information about the Vernier probeware used in this experiment, including tips and product specifications, visit **www.vernier.com/manuals** and download the appropriate user manual.

19. The experimental plans that your students submit for approval should list laboratory safety concerns, including chemical safety concerns, and specify how they will address these safety concerns during their investigations.

HAZARD ALERTS

The chemical safety signal words used in this experiment (**DANGER** and **WARNING**) are part of the Globally Harmonized System of Classification and labeling of Chemicals (GHS). Refer to the Safety Data Sheet (SDS) that came with the chemical for proper handling, storage, and disposal information. These can also be found online from the manufacturer. See Appendix F for more information.

Guaiacol solution, 0.2%: **DANGER**: Highly flammable liquid and vapor. Keep away from heat, sparks, open flames, and hot surfaces. May be harmful if swallowed. Causes skin and eye irritation. May cause drowsiness or dizziness. Avoid breathing mist, vapors or spray.

Hydrogen peroxide, 3%: **DANGER**: Causes skin and eye irritation.

Hydrogen peroxide, 0.02%: This chemical is considered nonhazardous according to GHS classifications. Treat all laboratory chemicals with caution. Prudent laboratory practices should be observed.

Sodium phosphate, dibasic, solution, 0.2 M: This chemical is considered nonhazardous according to GHS classifications. Treat all laboratory chemicals with caution. Prudent laboratory practices should be observed.

Sodium phosphate, monobasic, solution, 0.2 M: This chemical is considered nonhazardous according to GHS classifications. Treat all laboratory chemicals with caution. Prudent laboratory practices should be observed.