



EDVO-Kit: AP13

Enzyme Activity

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to introduce students to the concept of enzyme catalysis. Students will study the effect of various factors on the rate of reaction between an enzyme and its substrate. Students also learn to determine the optimal conditions under which the enzyme activity is maximized.

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THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.

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Experiment Components

- A Hydrogen peroxide solution
- B Guaiacol solution
- C Phosphate buffer pH 3
- D Phosphate buffer pH 7
- E Phosphate buffer pH 10
- F Phosphate buffer pH 14

Store entire experiment in the refrigerator.

This experiment is designed for 10 lab groups.

Requirements

- Turnip root
- Distilled or deionized water
- Serological pipets (2 ml and 5 ml)
- Pipet pumps or bulbs
- Erlenmeyer flask, 500 ml
- Spectrophotometer
- Water baths (for 4°, 37°, 60°, and 100° C)
- Filter paper and funnel
- Test tube racks
- Test tubes
- Thermometer
- Cheesecloth
- Parafilm
- Hot plate
- Timer or clock with second hand
- Lab permanent markers
- Ice
- Razor
- Goggles
- Blender

Background Information

Enzymes as Biological Catalysts

A biological **catalyst** is used in trace amounts and accelerates the rate of a biochemical reaction without being consumed or transformed during the reaction. The equilibrium constant of reactions are not altered by catalysts. Only the rate of approach to equilibrium is changed.

Reactions in cells are catalyzed by biological catalysts known as **enzymes**, which can accelerate reactions by as much as 10^{14} to 10^{20} times. Enzymes function best under mild physiological conditions of neutral pH, and temperatures of 37° C. Enzymes are generally very specific for the reactions they catalyze. Certain enzymes are regulated by intracellular concentrations of key metabolites that are not directly involved with the reaction they catalyze. This regulation (increase or decrease for an enzyme activity) is often regulated by a cell's physiological requirements at a given time. Enzymes that are regulated in this way are termed **allosteric**.

In 1897, Eduard Buchner demonstrated yeast cell free extracts to catalyze the fermentation of sugar to produce alcohol. In 1926, J.B. Sumner demonstrated that an enzyme was a protein. With the exception of specialized RNA molecules involved in RNA self-splicing and certain cyclodextrins, all naturally occurring enzymes to date are proteins. Thousands of different enzyme activities are known to have diverse and complex structures. There are however certain common structural features that are shared by all proteins.

Conformation of Proteins

Proteins consist of specific sequences of amino acid residues linked to each other by peptide bonds. The sequence of residues in a polypeptide chain is called **primary structure**. The unique amino acid sequences is the most important feature of primary structures. The sequence and distribution of amino acids have profound effects on the solubility, the three dimensional shape (**conformation**) and biological activity of a protein. (Chemical variety of amino acid side chain functional groups such as hydroxyl, carboxylic acid, amino, guanido, phenolic, sulfhydryl are largely responsible for the chemical activity, binding specificities, and electrical properties of proteins.) For example, the non-polar hydrocarbon groups of amino acids such as valine and alanine are important in maintaining the overall structure of a protein and creating the appropriate chemical environments within that is not in contact with the aqueous environment.

The backbone of the polypeptide chain consists of peptide bonds. The folding path of the backbone through space is called **secondary structure** of a protein. The folding patterns are complex, having bends, twists and spirals. Secondary structures are mainly determined by hydrogen bonds between backbone oxygens, nitrogens and hydrogens. Well known examples of secondary structure include **α -helices** and **β -pleated sheets**. Protein secondary structure is influenced by the type of amino acids present in that part of the polypeptide chain.

The complete three dimensional folding pattern of a polypeptide chain, including the positioning of the amino acid functional groups relative to each other, is called the **tertiary structure**. Examples of bonds which stabilize the tertiary structure of proteins are ionic bonds, hydrogen bonds, disulfide linkages, Van der Waals interactions, and hydrophobic interactions. The tertiary structure creates the three-dimensional crevices and pockets



Enzyme Activity

Background Information

which enable the protein to bind and react with substrate and other protein molecules. It also gives proteins unique conformation and affect solubility. Most importantly, the precise tertiary structure is absolutely necessary for the biological activity of proteins.

Many proteins consist of several polypeptide chains that are specifically associated with each other by non-covalent and covalent bonds. The three dimensional arrangement of polypeptide chains to each other in a protein is called **quaternary structure**. The individual polypeptide chains that make up the protein are often called subunits. The subunits of a protein can be identical, similar, or completely different from one another. Different subunits can be responsible for different functions within a protein.

Biological Activity of Enzymes

Certain proteins contain, as integral parts of their structure, chemical groups that are not amino acid residues but are absolutely required for biological activity. These groups include small organic molecules, such as certain vitamin derivatives and metal ions. Such moieties are called **prosthetic groups**. A well known prosthetic group is **heme**, which consists of an iron atom coordinated with nitrogen moieties that are part of complex organic ring compounds called **porphyrin**.

A protein that contains all its natural structural elements and possesses biological activity is called **native**. When a protein is unfolded, it no longer possesses biological activity even though the backbone and the amino acid groups remain intact. Unfolding can also cause subunit dissociation if there are no inter-subunit covalent links between them. Unfolded or inactive proteins are called **denatured**. Agents or conditions that denature enzyme structure or function will destroy their biological activity. Enzyme denaturation can be caused by high temperatures, extremes in pH, organic solvents, and repetitive cycles of freezing and thawing. Ionic detergents, such as (SDS) sodium dodecylsulfate, are potent protein denaturants that will bind to proteins and unfold their native forms. Other agents such as heavy metals, free radicals and peroxides disrupt protein structure by direct chemical reaction with the amino acid residues.

Measuring Enzyme Activity

The reactant molecule in an enzyme catalyzed reaction is called the **substrate**. The **substrate (S)** is transformed to **product (P)**. Before the enzyme can transform the substrate it must first bind to it. Only a relatively small portion of the enzyme molecule is involved with substrate binding and catalysis. This region is called the **active site**. The active site contains the critical amino acid residues and, if applicable, the **prosthetic groups** required for activity.

Initial binding is non-covalent and can be in rapid equilibrium. After productive binding has been achieved, the enzyme-substrate complex begins to generate product which is subsequently released. The **free enzyme (E)** can react with additional substrate and this reaction is repeated rapidly and effectively. The reaction is summarized using a single substrate, single product in a non-reversible reaction:

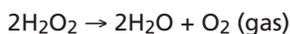


Background Information

Peroxidase is the enzyme used in this experiment.



For this investigation the specific reaction is as follows:



The appearance of product (P) or the disappearance of substrate (S) can be measured as a function of time during a reaction. One can measure the amount of product formed or the decrease in substrate at regular intervals (in this experiment at 30-sec intervals). This quantity can be plotted as a graph. Typical results are shown in Figure 1 which demonstrate the **rate of an enzymatic reaction**.

An enzymatic reaction measurement is referred to as an **assay**. At a fixed concentration and reaction conditions, an enzyme reaction rate can increase by higher substrate concentrations. The probability of forming ES complex increases with more substrate molecules present. Generally, the substrate concentration is thousands of times greater than the enzyme concentration for *in vitro* kinetic studies. At the early stages of such a reaction, the substrate concentration is in great excess and the rate is approximately linear per unit of time and is termed the **initial velocity (v) or initial rate** of the reaction. The characteristics of the enzyme molecule determine the initial velocity. It will always remain the same for an enzyme as long as the substrate is present in excess, the products are not inhibitory and the pH and temperature remain constant.

$$\frac{[S]_1 - [S]_2}{T_1 - T_2}$$

In the above equation, $[S]_1$ is the molar concentration of substrate at some initial time T_1 , and $[S]_2$ is the substrate concentration at a later time T_2 . Note that the concentration of substrate decreases with time and the concentration of product increases with time. Graphically, this can be represented with the substrate concentration plotted on the y-axis and time on the x-axis. The decrease in the substrate concentration with time will generate a curve. The rate of decrease is fastest at the earliest time points of the reaction since the substrate concentration is comparatively higher. The rate of decrease diminishes at later times because the substrate concentration is lower and the reaction is slower (**Figure 1**). Within short intervals, there will be sections of the curve that are approximately linear and the rate of the reaction can be measured. At some substrate concentration, all the enzyme molecules are bound to substrate and are involved in some stage of the catalytic reaction. Under these conditions the enzyme is saturated with substrate and no increase in reaction velocity.

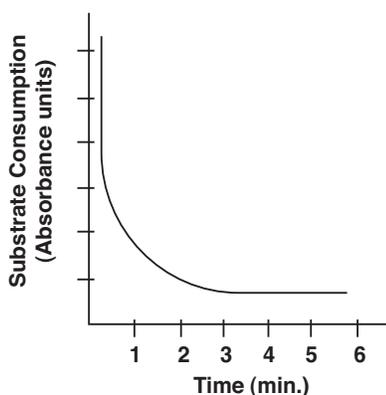


Figure 1



Background Information

The initial reaction rate can also be expressed in terms of the appearance of product. To determine the rate of the reaction, pick any two points on the straight-line portion of the graph curve (**Figure 2**). The amount of product formed between two points divided by the difference in time between the two points will be the rate of the reaction. It can be expressed as $\mu\text{moles product/sec}$.

$$\frac{[P]_2 - [P]_1}{T_2 - T_1}$$

There is no product formed at time 0. 10 μmoles have been formed after 30 seconds; 20 μmoles after 60 seconds; 30 μmoles after 90 seconds. For the initial period, the rate of this reaction could be stated as 20 μmoles of product formed per minute. Typically, less additional μmoles of product are formed by the second, third and fourth minute (**Figure 2**). For each successive minute after the initial 1.5 minutes, the amount of product formed is less than in the preceding minute.

As the illustration of Figure 2:

$$\frac{30 \mu\text{moles} - 10 \mu\text{moles}}{90 \text{ seconds} - 30 \text{ seconds}} = \frac{20}{60} = 0.33 \mu\text{moles/sec}$$

In this experiment, an indicator for oxygen will be used. The compound Guaiacol has a high affinity for oxygen. When in solution, Guaiacol binds instantly with oxygen to form tetraguaiacol, turning the product into a brownish solution. The greater the amount of oxygen produced, the darker brown the product solution will become.

Baseline is a line that is used as a point of reference. In this investigation the term "baseline" is used to establish a standard for a reaction. Thus when manipulating components of a reaction (enzyme concentration, pH, temperature), students will use the baseline as their reference to help understand what occurred in the reaction.

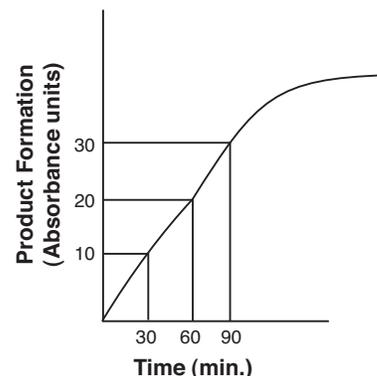


Figure 2

Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE

The objective of this experiment is to introduce students to the concept of enzyme catalysis. Students will study the effect of various factors on the rate of reaction between an enzyme and its substrate. Students also learn to determine the optimal ranges under which the enzyme activity is maximized.

EXPERIMENT OVERVIEW

In this experiment, students will first develop a method for measuring peroxidase in turnips and determine a baseline. Then they will study the effect of various factors such as enzyme concentration, pH, and temperature on the activity of the turnip peroxidase enzyme.

LABORATORY SAFETY GUIDELINES

1. Always wear gloves and goggles when working in the laboratory.
2. Exercise caution when working in the laboratory as you will be using equipment that can be dangerous if used incorrectly.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Always wash hands thoroughly with soap and water after working in the laboratory.
5. If you are unsure of something, ASK YOUR INSTRUCTOR!



LABORATORY NOTEBOOKS

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you will be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results - does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Experimental Procedures**A few notes before the start of the experiment:**

- In this experiment, students will be graphing various charts to demonstrate the effect of different abiotic and biotic factors on enzyme activity of Peroxidase. You may do this by hand, using the graph paper provided, or on a spreadsheet program such as Excel.
- In Investigation I, students will construct a baseline activity of Turnip Peroxidase, and then compare this baseline activity with enzyme activity under different conditions in Investigations II and III.

Investigation I: Determining a Baseline by Measuring Peroxidase in Turnips**A. SETTING UP THE REACTIONS**

1. Turn on the spectrophotometer and adjust the Absorbance to 500 nm. Allow the spectrophotometer to warm up for approximately 15 minutes.
2. Using a permanent marker, label one test tube "S" (Substrate) and label the other test tube "E" (Enzyme). Also label one additional test tube as "B" (Blank).
3. Add the following components to the tubes as summarized in Chart 1. Use a FRESH micropipet tip for each transfer of the chemicals. Cover the test tubes and gently mix well.

Chart 1 – Summary of Substrate and Enzyme Tubes					
Tube	Hydrogen Peroxide solution	Guaiacol solution	Turnip Peroxidase	Distilled or deionized water	Final Volume
S (Substrate)	0.15 ml	0.1 ml	-----	3.5 ml	3.75 ml
E (Enzyme)	-----	-----	0.75 ml	3.0 ml	3.75 ml
Blank	-----	-----	-----	7.5 ml	7.5 ml

Investigation I: Determining a Baseline by Measuring Peroxidase in Turnips

B. DATA COLLECTION

1. Be sure the spectrophotometer is set at 500 nm. Spectral readings can now be taken. Depending on the spectrophotometer, you may be able to insert your test tubes directly into the instrument. Otherwise transfer the entire enzyme reactions in appropriate sized tubes provided by your instructor.
2. Zero the instrument with the tube B solution (Blank) according to your instructor's directions. The instrument should read 0 absorbance with the blank solution (no color).
3. Remove the blank.
4. When ready to begin a reaction, carefully combine the contents of tube S and tube E. Cover the test tube with a piece of Parafilm. Quickly invert to mix well.
5. Place the test tube in the spectrophotometer cuvette holder, and immediately start timing.
6. Measure and record the absorbance every 30 seconds for the next 5 minutes in Table 1 below.
 - If you feel comfortable removing the tube from the spectrophotometer cell holder and putting it back before the 30 second interval elapses, do so and observe the color change within the 5 minute period.
 - Rotate the tube before each reading. Record the observed color at the end of every 30 second interval.
 - A cell phone and/or camera are excellent ways to record color change.
7. Repeat the above steps to determine the absorbance of different enzyme concentrations (1/2 X and/or 2X) on peroxidase activity.
8. Record the results in the table.

Time (sec)	Absorbance 1/2 X Enzyme	Absorbance 1X Enzyme	Absorbance 2X Enzyme
0			
30			
60			
90			
120			
150			
180			
210			
240			
270			
300			

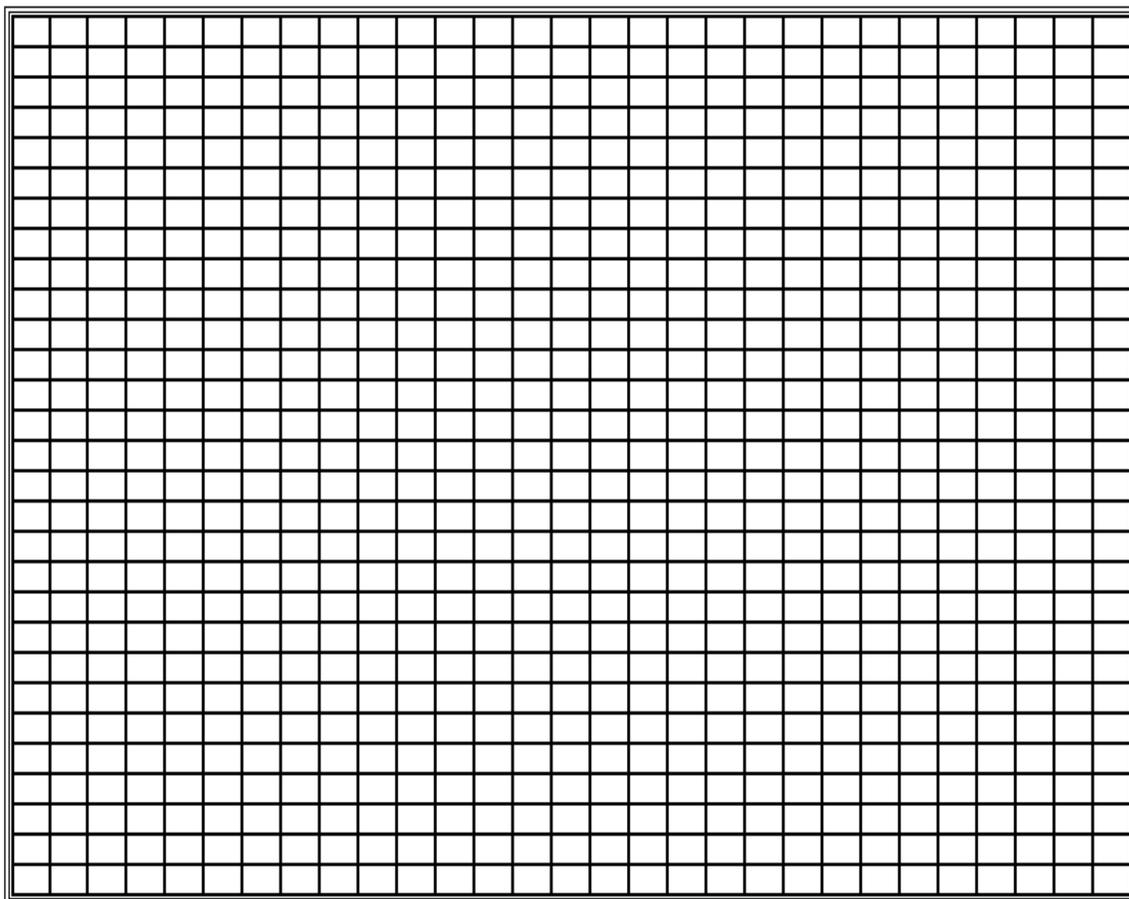
Table 1. Absorbance from turnip peroxidase baseline.



Investigation I: Determining a Baseline by Measuring Peroxidase in Turnips

C. GRAPHING AND CALCULATING THE REACTION RATE

1. You should have three sets of “absorbance vs. time” data from Investigation I, one from each enzyme concentration.
2. Plot the data from three sets on the same graph using the graph paper provided below. Extrapolate a straight line through the linear portion of the reaction curve.



3. Next, calculate the slopes of each line to determine the enzyme activity. The slope of the linear portions of the curves is a measure of enzymatic activity.

Question 1: Based on the data obtained from the graph, determine which concentration of enzyme is most suitable for the analysis. Explain.

Enzyme Activity—Opportunities for Inquiry

- Investigations II and III are suggestions for the student-directed lab activities. Students investigate the effects of pH and temperature on the rate of the peroxidase-catalyzed reaction.
- Students are encouraged to conduct several trials to determine the enzymatic activity of Peroxidase in response to such environmental conditions. Analyze and graph the result for each trial.



Investigation II: Determining the Effect of pH on Enzymatic Activity**A. SETTING UP THE REACTIONS:**

- Using a permanent marker, label four pairs of tubes with each pair containing the different pH buffers, as follows:

Set 1: pH3 "S" and pH3 "E"
 Set 2: pH7 "S" and pH7 "E"
 Set 3: pH10 "S" and pH10 "E"
 Set 4: pH14 "S" and pH14 "E"

- Add the following components to the tubes as summarized in Chart 2. Use a FRESH micropipet tip for each transfer of the chemicals. Cover the test tubes and gently mix well.

Note: Add the different pH buffer to the four "E" tubes. Do not add the different pH buffer to the four "S" (substrate) tubes!

Chart 2 – Summary of Substrate and Enzyme Tubes with Different pH Buffers						
Tube	Hydrogen Peroxide solution	Guaiaicol solution	Turnip Peroxidase	pH Buffer (various pH)	Distilled or deionized water	Final Volume
pH 3 "S"	0.15 ml	0.1 ml	-----	-----	3.5 ml	3.75 ml
pH 3 "E"	-----	-----	0.75 ml	3.0 ml (pH 3)	----	3.75 ml
pH 7 "S"	0.15 ml	0.1 ml	-----	-----	3.5 ml	3.75 ml
pH 7 "E"	-----	-----	0.75 ml	3.0 ml (pH 7)	----	3.75 ml
pH 10 "S"	0.15 ml	0.1 ml	-----	-----	3.5 ml	3.75 ml
pH 10 "E"	-----	-----	0.75 ml	3.0 ml (pH 10)	----	3.75 ml
pH 14 "S"	0.15 ml	0.1 ml	-----	-----	3.5 ml	3.75 ml
pH 14 "E"	-----	-----	0.75 ml	3.0 ml (pH 14)	----	3.75 ml

Investigation II: Determining the Effect of pH on Enzymatic Activity

B. DATA COLLECTION

- Remember to blank the spectrophotometer with the blank solution at 500 nm. The instrument should read 0 absorbance. No color change should occur. Remove the blank.
- When ready to begin the reaction, carefully combine the contents of tube S and tube E containing pH 3 buffer. Cover the test tube with a piece of Parafilm. Quickly invert to mix well.
- Place the test tube in the spectrophotometer cuvette holder, and immediately start timing.
- Measure and record the absorbance every 30 seconds for the next 5 minutes in Table 2 below.

Note:

- If you feel comfortable removing the tube from the spectrophotometer cell holder and putting it back before the 30 second interval elapses, do so and observe the color change within the 5-minute period.*
 - Rotate the tube before each reading. Record the observed color at the end of every 30-second intervals.*
 - A cell phone and/or camera are excellent ways to record color change.*
- Repeat the above steps for the remaining 3 pairs of tubes containing buffers at pH 7, pH 10, and pH 14.
 - Record the results in the table.

Time (sec)	Absorbance at pH 3	Absorbance at pH 7	Absorbance at pH 10	Absorbance at pH 14
0				
30				
60				
90				
120				
150				
180				
210				
240				
270				
300				

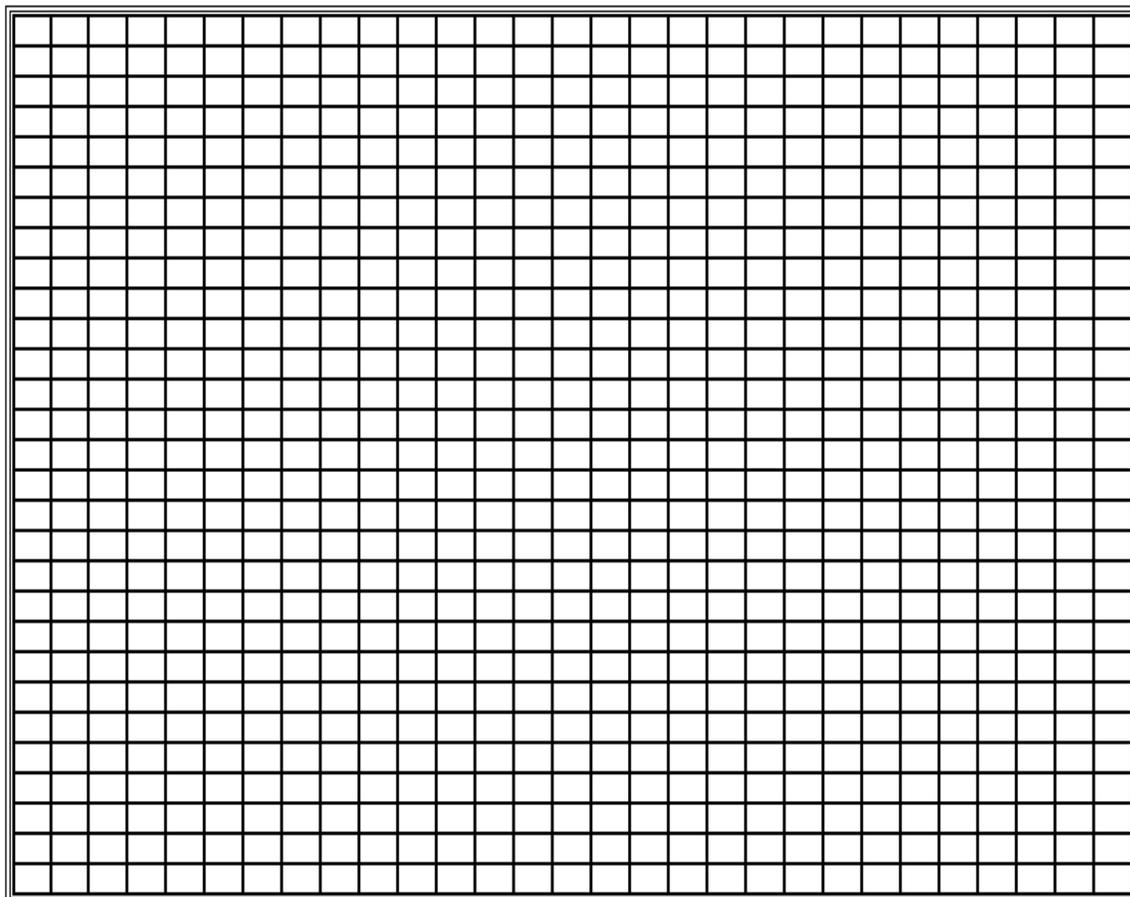
Table 2. Absorbance at different pH.



Investigation II: Determining the Effect of pH on Enzymatic Activity

C. GRAPHING THE ABSORBANCE AT DIFFERENT pH

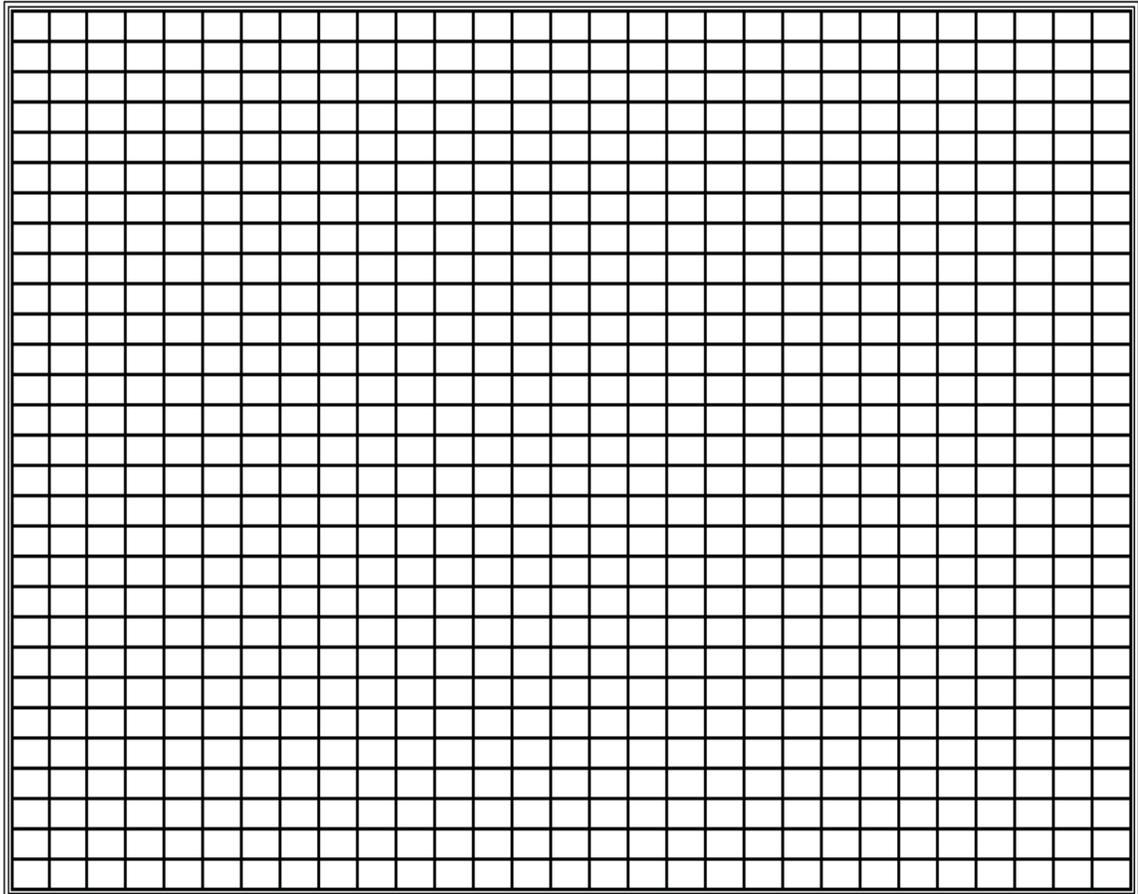
1. You should have four sets of "absorbance vs. time" data from Investigation II, one from each pH.
2. Plot the data from four sets on the same graph using the graph paper provided below.



3. Next, calculate the slopes of each line to determine the enzyme activity.

Investigation II: Determining the Effect of pH on Enzymatic Activity

4. Plot another graph using the graph paper below to show correlation between enzyme activity and pH. (You should have four points for this chart, try to connect the points to form a smooth curve for these sets of data.)



Question 2: At what pH is the turnip peroxidase enzyme most active at?

Investigation III: Determining the Effect of Temperature on Enzymatic Activity**A. SETTING UP THE REACTIONS**

- Using a permanent marker, label four sets of pairs of tubes, as follows

Set 1: 4 °C "S" and 4 °C "E"
 Set 2: 37 °C "S" and 37 °C "E"
 Set 3: 60 °C "S" and 60 °C "E"
 Set 4: 100 °C "S" and 100 °C "E"

- Add the following components to the tubes as summarized in Chart 1. Use a FRESH micropipet tip for each transfer of the chemicals. Cover the test tubes and gently mix well.

Note: Each of the four sets will consist of a Substrate and an Enzyme tube for a total of 8 tubes or 4 pairs. Each set will be treated at different temperature settings to observe the effect of temperature on the enzymatic activity of Peroxidase.

Chart 3 – Summary of Substrate and Enzyme Tubes

Tube (4 of each)	Hydrogen Peroxide solution	Guaiacol solution	Turnip Peroxidase	Distilled or deionized water	Final Volume
S (Substrate)	0.15 ml	0.1 ml	-----	3.5 ml	3.75 ml
E (Enzyme)	-----	-----	0.75 ml	3.0 ml	3.75 ml

B. DATA COLLECTION

- Remember to blank the spectrophotometer with the blank solution at 500 nm. Your reading should read 0, and no color changes should occur. Remove the blank.
- Incubate the 1st set of Substrate and Enzyme tubes labeled 4° C "S" and 4° C "E" in a 4° C ice water bath for 10 minutes.
- After the incubation time is up, carefully combine the contents of tube S and tube E. Cover the test tube with a piece of Parafilm. Quickly invert to mix well.
- Place the test tube in the spectrophotometer cuvette holder, and immediately start timing.

Investigation III: Determining the Effect of Temperature on Enzymatic Activity

- Measure and record the absorbance every 30 seconds for the next 5 minutes in Table 3 below.

Note:

- If you feel comfortable removing the tube from the spectrophotometer cell holder and putting it back before the 30 second interval elapses, do so and observe the color change within the 5-minute period.*
 - Rotate the tube before each reading. Record the observed color at the end of every 30-second interval.*
 - A cell phone and/or camera are excellent ways to record color change.*
- Repeat the above steps for the remaining 3 pairs of tubes at 37 °C, 60 °C, and 100 °C.
 - Record the results in the table.

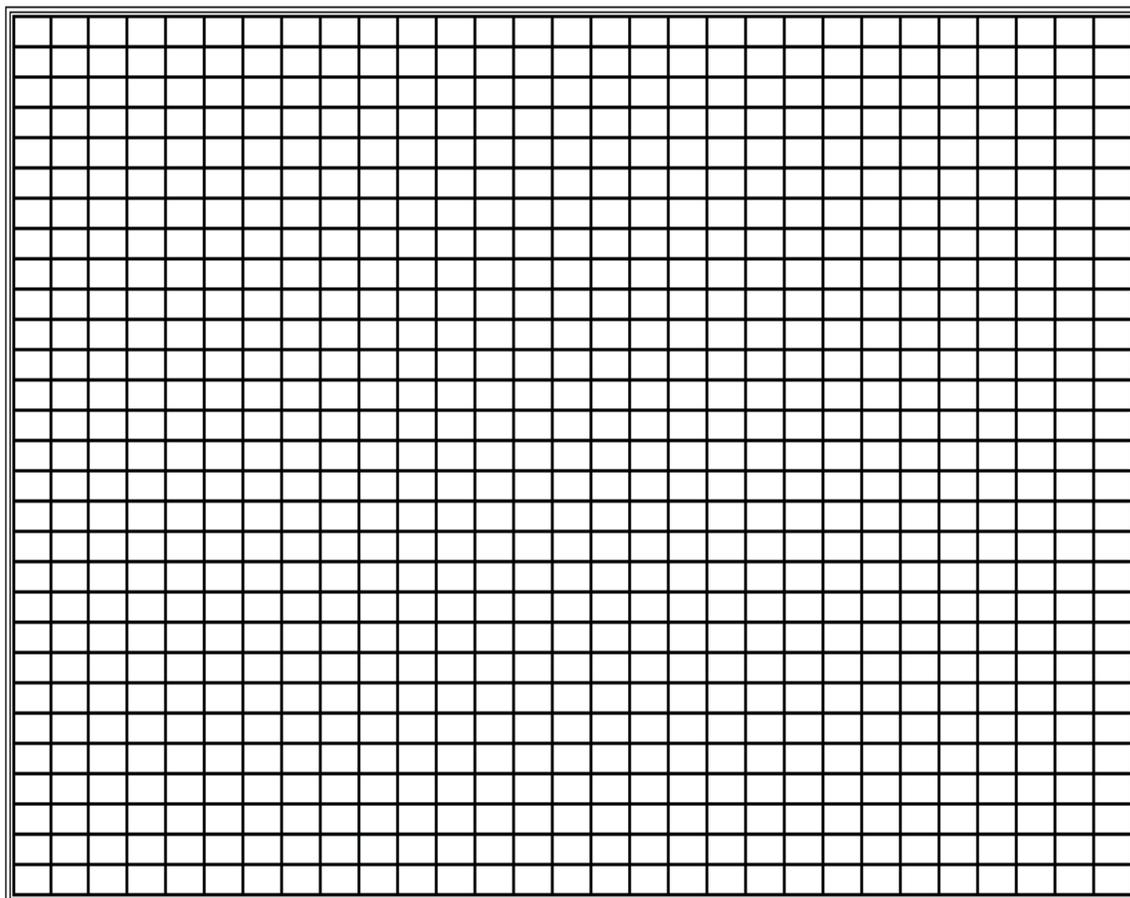
Time (sec)	Absorbance at 4° C	Absorbance at 37° C	Absorbance at 60° C	Absorbance at 100° C
0				
30				
60				
90				
120				
150				
180				
210				
240				
270				
300				

Table 3. Absorbance at different temperatures.

Investigation III: Determining the Effect of Temperature on Enzymatic Activity

C. GRAPHING THE ABSORBANCE AT DIFFERENT TEMPERATURE

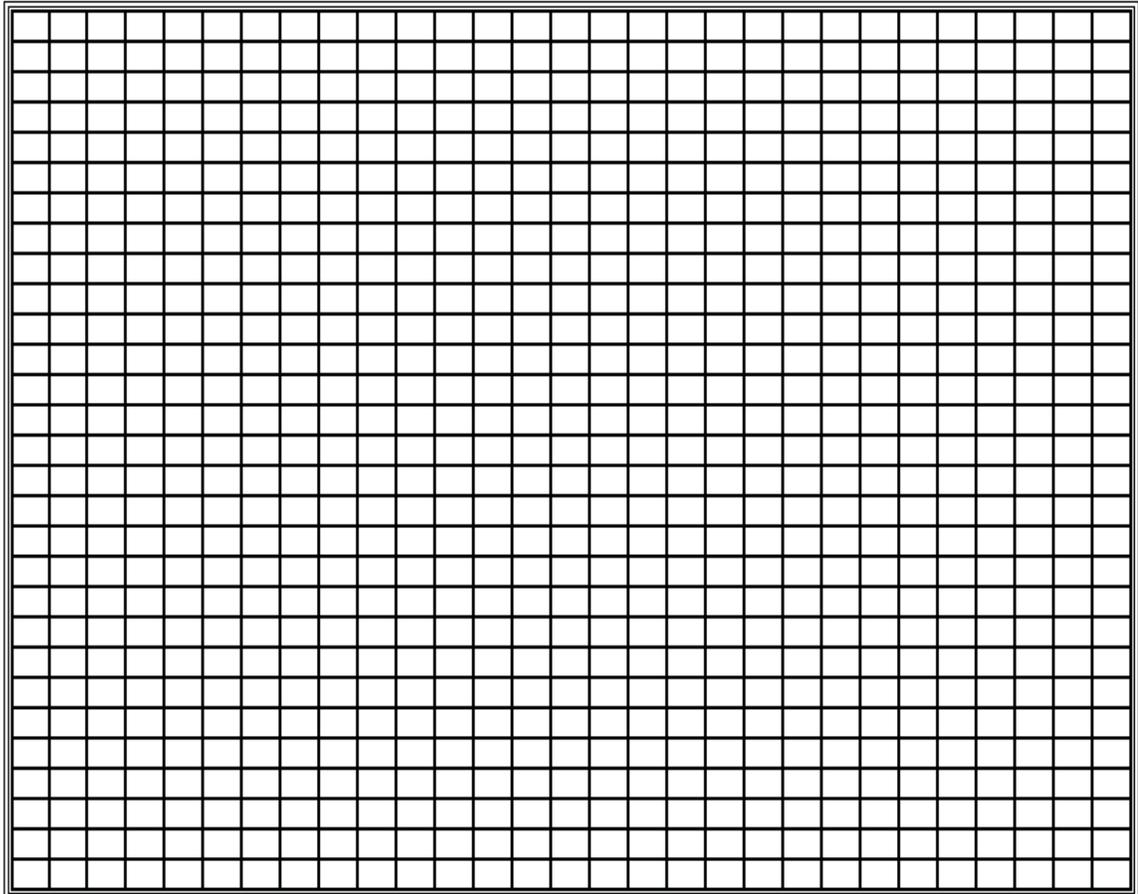
1. You should have four sets of "absorbance vs. time" data from Investigation III, one from each temperature.
2. Plot the data from four sets on the same graph using the graph paper provided below.



3. Next, calculate the slopes of each line to determine the enzyme activity.

Investigation III: Determining the Effect of Temperature on Enzymatic Activity

4. Plot another graph using the graph paper below to show correlation between enzyme activity and temperatures. (You should have four points for this chart, try to connect the points to form a smooth curve for these sets of data.)



Question 3: At what temperature is the turnip peroxidase enzyme most active at?

Experiment Results and Analysis

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results - does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Study Questions

1. Why was the standardization of enzyme concentration performed?
2. What is the enzyme in this reaction?
3. What is the substrate in this reaction?
4. What are the products in this reaction?
5. What is the function of the Guaiacol?
6. Explain why the color intensity of the peroxide assays increased with time?
7. What makes the rate of a reaction of an enzymatic reaction decrease?
8. Assuming optimal reaction conditions (pH, temperature, etc.), how could you increase the rate of the reaction other than increasing the substrate concentration?

Instructor's Guide

Notes to the Instructor

OVERVIEW OF LABORATORY INVESTIGATIONS

The "hands-on" laboratory experience is a very important component of the science courses. Laboratory experiment activities allow students to identify assumptions, use critical and logical thinking, and consider alternative explanations, as well as help apply themes and concepts to biological processes.

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EDVOTEK experiments have been designed to provide students the opportunity to learn very important concepts and techniques used by scientists in laboratories conducting biotechnology research. Some of the experimental procedures may have been modified or adapted to minimize equipment requirements and to emphasize safety in the classroom, but do not compromise the educational experience for the student. The experiments have been tested repeatedly to maximize a successful transition from the laboratory to the classroom setting. Furthermore, the experiments allow teachers and students the flexibility to further modify and adapt procedures for laboratory extensions or alternative inquiry-based investigations

ORGANIZING AND IMPLEMENTING THE EXPERIMENT

Class size, length of laboratory sessions, and availability of equipment are factors, which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

If you do not find the answers to your questions in this section, a variety of re-sources are continuously being added to the EDVOTEK web site.

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In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

This investigation requires approximately three to four 45-minute lab periods. Students can work in pairs or small groups to accommodate different class sizes. Additional time outside of class would be required to complete data tables and Excel and graphing analysis of data collected.



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Please have the following information ready:

- Experiment number and title
- Kit lot number on box or tube
- Literature version number (in lower right corner)
- Approximate purchase date

Visit the EDVOTEK web site often for updated information.

Pre-Lab Preparations

A. PREPARATION OF THE TURNIP EXTRACT

1. Exercise care when using a knife to peel and cut the turnip. Remove 20 g of peeled turnip from the inner portion of the vegetable. Cut the turnip into small pieces.
2. Blend it thoroughly in a blender with 500 ml distilled water for 1 minute, using the pulse option.
3. Filter the enzyme extract into a beaker through 3 layers of cheesecloth and place the filtered extract on ice. This suspension is the turnip extract and contains the enzyme peroxidase at 1X concentration.
4. Repeat steps 1-3 using different weights of turnip to prepare two additional enzyme extracts at $\frac{1}{2}$ X and 2X concentrations, as follows:
 - a. $\frac{1}{2}$ X: use 10 g of peeled turnip in a final volume of 500 ml distilled water.
 - b. 2X: use 40 g of peeled turnip in a final volume of 500 ml distilled water.
5. Dispense 10 ml of 1X enzyme per tube. Label these tubes "1X Enzyme."
6. Dispense 1 ml of $\frac{1}{2}$ X enzyme per tube. Label these tubes " $\frac{1}{2}$ X Enzyme."
7. Dispense 1 ml of 2X enzyme per tube. Label these tubes "2X Enzyme."
8. Store the extract in a dark bottle and refrigerate.

Note: The activity of the turnip extract will vary from experiment to experiment, depending on the size and age of the turnip.

B. PREPARATION OF OTHER SOLUTIONS

1. **Hydrogen Peroxide Solution:**
 - a. To a small beaker containing 43.5 ml of distilled or deionized water, add all of the contents of 3% Hydrogen Peroxide solution. Mix well.
 - b. Dispense 4 ml of the diluted hydrogen peroxide into each tube.
 - c. Label these 10 tubes "Hydrogen Peroxide" and distribute one tube per student group.
 - d. Store in a dark bottle and protect from heat and light.

Pre-Lab Preparations

2. Guaiacol Solution*:

- To a small beaker containing 45 ml of distilled or deionized water, add all of the contents of Guaiacol solution. Mix well.
- Dispense 4 ml of the diluted Guaiacol Solution into each tube.
- Label these 10 tubes "Guaiacol" and distribute one tube per student group.
- Store in a dark bottle and protect from heat and light.

3. Reaction Buffers:

Dispense 3.2 ml of each of the phosphate buffers (pH 3, pH 7, pH 10, pH 14) per group. Label these tubes accordingly.

- * **Note:** *Guaiacol has an aromatic odor. However, it is considered toxic and may be irritating to the eyes, nose and throat. Exercise care when preparing the Guaiacol solution. Refer to the MSDS for additional safety and handling information.*

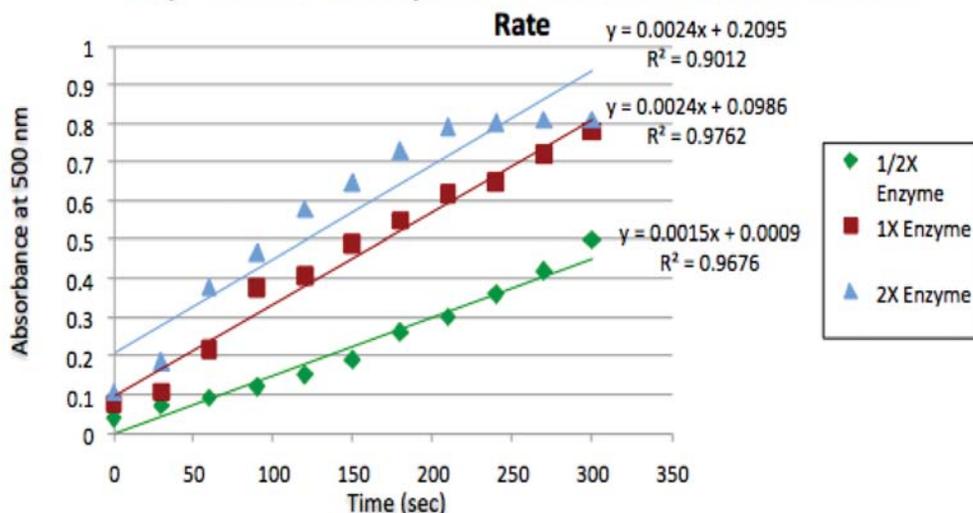
Each lab group should have the following materials:

- 3 tubes containing 3 different enzyme extracts at different concentrations (1X, 1/2X, and 2X)
- 1 tube containing Hydrogen Peroxide
- 1 tube containing Guaiacol
- 4 tubes containing 4 different phosphate buffers at different pH (pH 3, pH 7, pH10, pH 14)

Experiment Results and Analysis

INVESTIGATION I: DEVELOPING A METHOD FOR MEASURING PEROXIDASE IN PLANT MATERIAL AND DETERMINING A BASELINE

Graph I - Effect of Turnip Peroxidase Concentration on Reaction



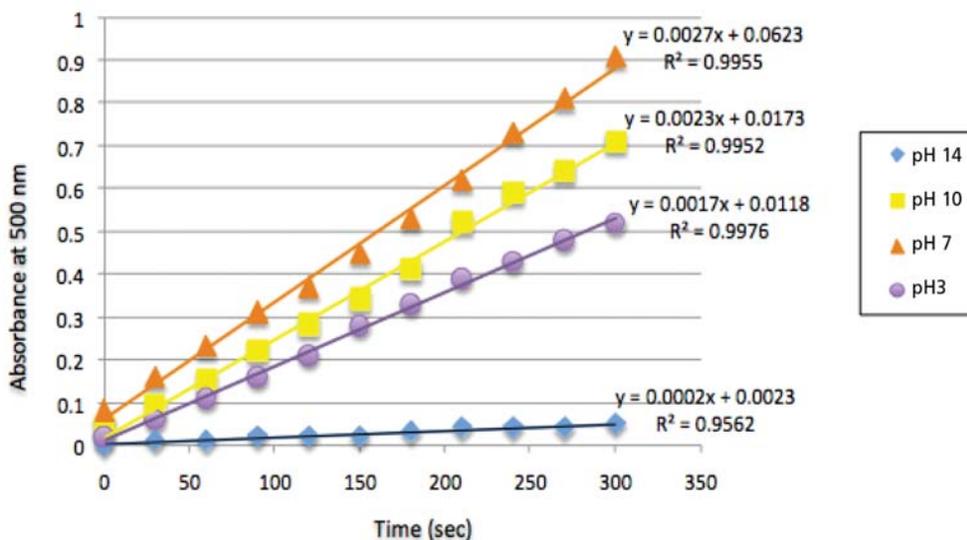
Question 1: Based on the data obtained from the graph, determine which concentration of enzyme is most suitable for the analysis. Explain.

Answer: The enzyme is most suitable for the analysis at the concentration of 1X. The graph showed that three enzyme concentrations produced visible reaction. The 1/2 X was linear, but the slope is very shallow. The 1X was also linear, but with a steeper slope. The 2X was linear for the first portion, and levels off at the end. This leveling off is due to the enzyme exhausting all of the peroxide in the solution. In our experiment, the most appropriate concentration is the 1X, as it had the greatest reaction rate without running the reaction to completion in the allotted time.

Experiment Results and Analysis

INVESTIGATION II: DETERMINING THE EFFECT OF pH ON ENZYMATIC ACTIVITY

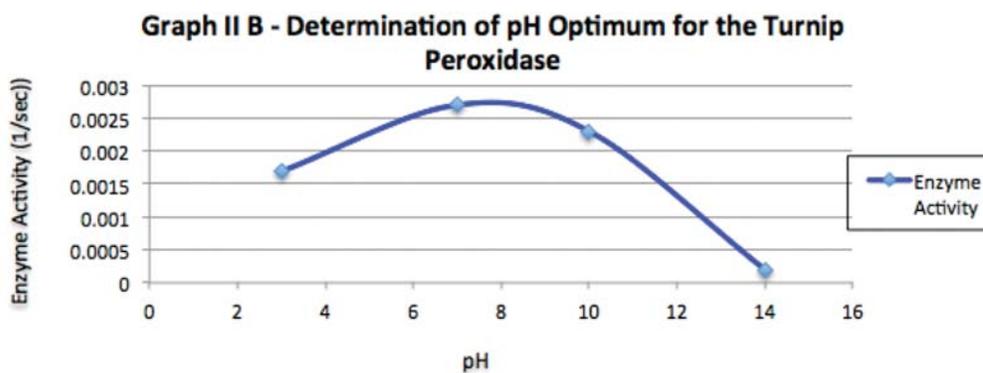
Graph II A - Effect of pH on Turnip Peroxidase Enzyme



Experiment Results and Analysis

II B - Determination of pH Optimum for the Turnip Peroxidase Enzyme:
The slopes are graphed against pH to relate the enzyme activity to pH

pH	3	7	10	14
Enzyme Activity	0.0017	0.0027	0.0023	0.0002

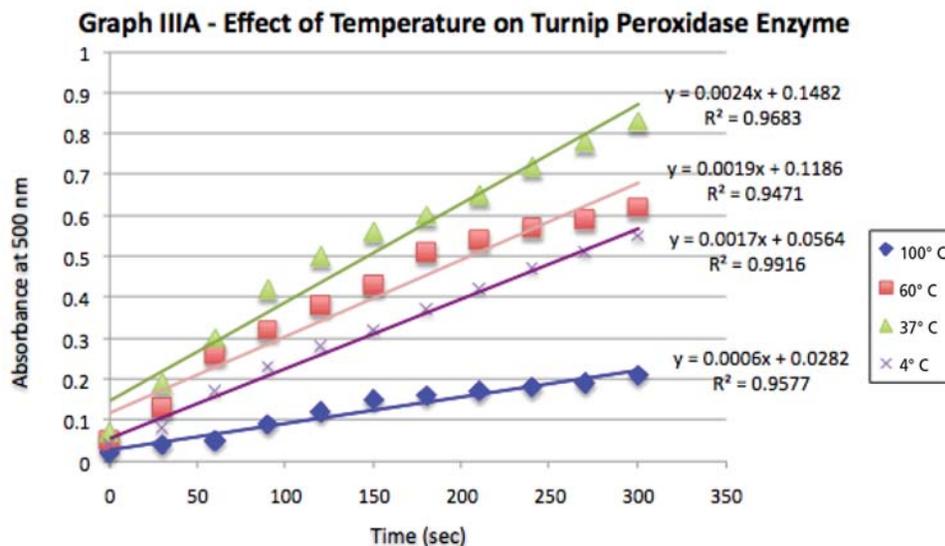


Question 2: At what pH is the turnip peroxidase enzyme most active at?

Answer: The optimal pH for the turnip peroxidase enzyme is around 6.8 – 7.0.

Experiment Results and Analysis

INVESTIGATION III: DETERMINING THE EFFECT OF TEMPERATURE ON ENZYMATIC ACTIVITY



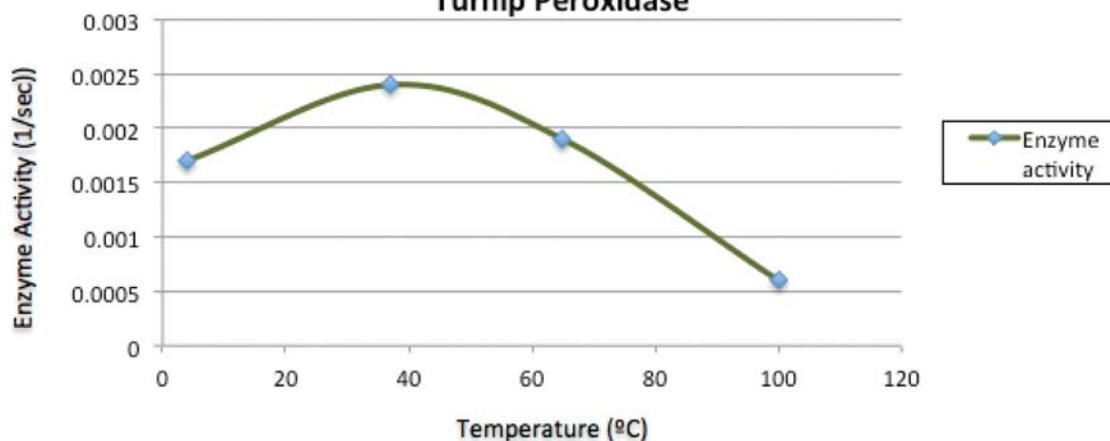
Instructor's Guide

Experiment Results and Analysis

III B - Determination of Temperature Optimum for the Turnip Peroxidase Enzyme:
The slopes are graphed against Temp. to relate the enzyme activity to Temp.

Temperature	4° C	37° C	60° C	100° C
Enzyme Activity	0.0017	0.0024	0.0019	0.0006

Graph III B - Determination of Temperature Optimum for the Turnip Peroxidase



Question 3: At what temperature is the turnip peroxidase enzyme most active at?

Answer: The optimal temperature for the turnip peroxidase enzyme is around 42° C – 45° C.

**Please refer to the kit
insert for the Answers to
Study Questions**

Material Safety Data Sheets

Full-size (8.5 x 11") pdf copy of MSDS is available at www.edvotek.com or by request.

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IDENTITY (As Used on Label and List) Hydrogen Peroxide, Stabilized	Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.																																					
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Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code) 1121 5th Street NW Washington DC 20001	Emergency Telephone Number 202-370-1500 Telephone Number for information 202-370-1500 Date Prepared 11-17-11 Signature of Preparer (optional)																																					
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Work/Hygienic Practices Avoid inhalation. Keep away from incompatibilities and combustible material.																																						

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Steps to be Taken in case Material is Released for Spilled Wear suitable protective clothing. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Avoid breathing dust.																																						
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Precautions to be Taken in Handling and Storing Keep container tightly closed in a dry and well-ventilated place. Store under inert gas. Air and light sensitive																																						
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