

WARD'S

AP Biology Lab 2

Enzyme Catalysis

Lab Activity

Student Study Guide

BACKGROUND



Enzyme: a protein molecule that catalyzes specific metabolic reactions without itself being permanently altered or destroyed.

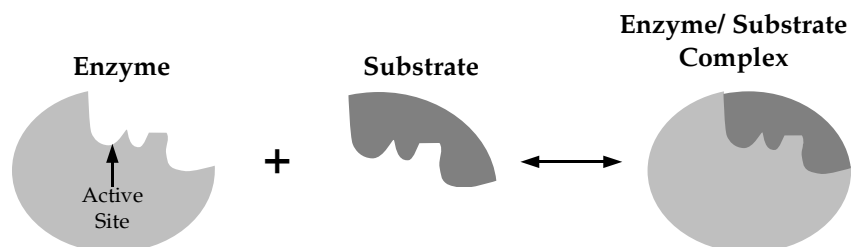
Substrate: a substance acted upon by an enzyme.

Enzyme Structure and Function

Certain chemical reactions, necessary to sustain the life of a cell, must be carried out quickly and efficiently. A cell cannot depend on the possibility that random events will cause the necessary reaction to occur that will keep it alive; therefore, cells employ a particular molecule, called an enzyme, to aid in the process. For example, a disaccharide, a simple molecule that is a carbohydrate common in cells, is composed of two units called monosaccharides. Monosaccharides are a major source of energy for all cells; disaccharides and larger molecules called polysaccharides, which are made up of three or more monosaccharides, are commonly taken in as nutrients. Eventually the molecules would break down into monosaccharides on their own, but not in time to support a cell's life. Therefore, cells utilize an enzyme that can cause a particular molecule to break apart or come back together. These enzymes are complex proteins that consist of one or many polypeptide chains, forming a shape crucial to the kinetics of enzyme-substrate interactions.

Enzyme-Substrate Kinetics

For an enzyme to be specific, it must fit over a molecule. The portion of the enzyme that fits over the molecule is called the active site. The molecule that the active site is reacting with is called the substrate. When the enzyme is reacting with the substrate, a complex is formed. This interaction can be expressed as:



Some enzymes change shape after binding to a substrate, improving the "fit" between the enzyme and the substrate. This new formation is called the "induced fit model".



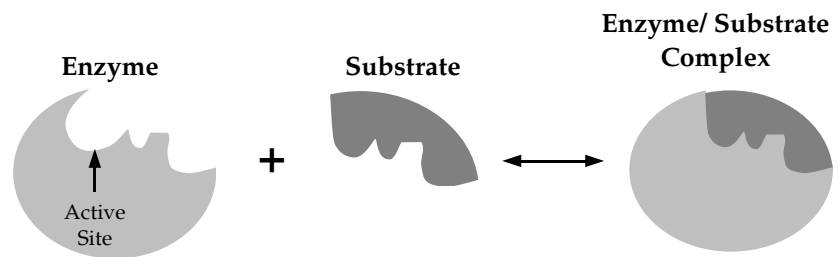
DID YOU KNOW?

There are over 2000 known enzymes, each of which is involved with one specific chemical reaction.



DID YOU KNOW?

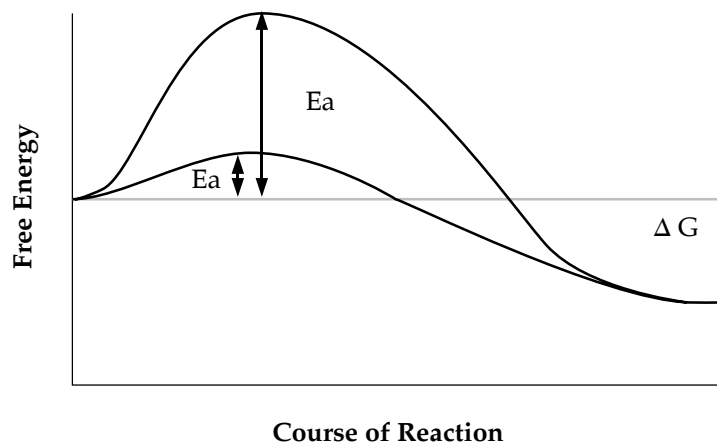
Thermolabile enzymes, such as those responsible for the color patterns in Siamese cats and the color camouflage of the Arctic fox, work better at lower temperatures.



Note that in the equation, the arrows go in both directions. This illustrates the principle of reversibility, meaning that an enzyme can break down substrates, as well as put the substrates back together to reform the product.

Roles of Enzymes

The enzyme-driven reactions that occur spontaneously under the proper circumstances occur more quickly when enzymes act as catalysts. The enzymes allow these reactions to occur more efficiently by lowering the activation energy required to drive the reaction. The graph below illustrates the differences between the amounts of activation energy (represented by E_a); the enzyme-catalyzed reaction has a lower activation energy than an uncatalyzed reaction. Note that there is no difference in the amount of free energy (ΔG).



Enzyme Regulation

Enzyme reactions can be affected by a variety of conditions, some of which are environmental and include variables such as temperature, pH, and the concentration of the enzyme or substrate. Other regulatory mechanisms, however, function only with specific enzymes to alter the enzymes' action, resulting in an increased or decreased reaction rate.

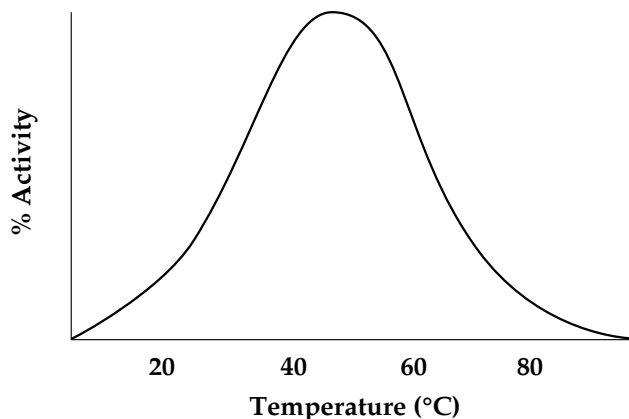


DID YOU KNOW?

Enzymes can act very rapidly. For example, carbonic anhydrase causes chemicals within the human body to react ten times faster than without the enzyme present.

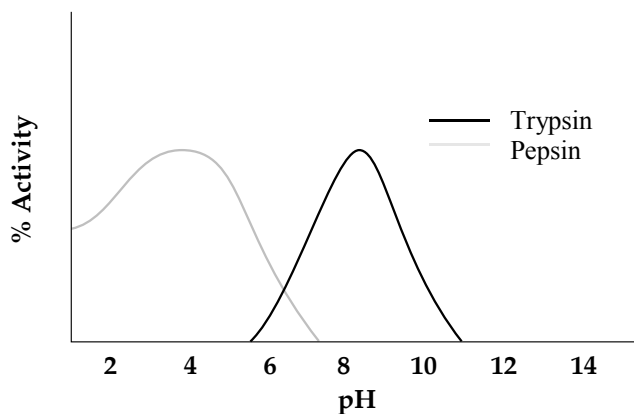
Effect of Temperature

Raising the temperature of any substance will cause a rise in its average kinetic energy, because the heat in any given system is expressed as molecular motion. The more heat that is added, the more molecular motion will occur. As a general rule, an increase of 10°C doubles the rate of most chemical reactions. This is also true for enzymes. Enzymes are proteins, however, and so are subject to heat-induced destruction of their tertiary structure. When the shape of an enzyme is altered, it is called denaturation, and the enzyme becomes inoperative.



Effect of pH

The relative acidity or alkalinity of an enzyme's surroundings also influences its activity. Most enzymes require a neutral pH, while some work best in conditions that are extremely acidic or alkaline. An enzyme's preference depends on the R groups of the active sites' amino acids. Because many of the R groups ionize when dissolved in water, enzymes are charged at these groups. If the charged R group is an integral part of the active site, the pH of the environment, which is related to the concentration of H^+ ions, determines the charges that the enzyme can carry. For an enzyme carrying many negatively charged R groups at the active site, an acidic pH will neutralize the R groups, rendering the active site inactive. Some enzymes will not be active until negative charges are neutralized or, conversely, until +R groups are neutralized in a basic environment. Two examples are illustrated in the graph below: pepsin, a hydrolytic stomach enzyme, and trypsin, an intestinal enzyme.



**Exergonic reactions:**

Biochemical reactions that release or generate energy and are thus capable of proceeding spontaneously.

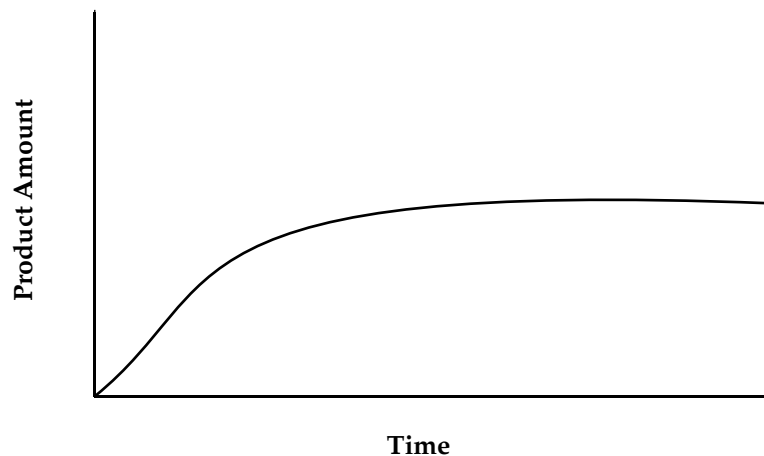
Endergonic reactions:

Biochemical reactions that require energy and thus cannot proceed spontaneously.

Effect of Concentration

Since enzyme reactions are reversible, it could be speculated that the enzymes could be caught in a loop, forming products, breaking them down, and reassembling them, over and over. Yet enzymes follow the Law of Mass Action: the direction taken by an enzyme-catalyzed reaction is directly dependent on the relative concentration of enzyme, substrate, and product. When there is a great deal of substrate and little product, the reaction will form more product. Conversely, when there is a great deal of product and little enzyme, the reaction will form more substrate.

There are two situations where the Law of Mass Action is circumvented. When the product is immediately metabolized or transported away from the enzyme, the product concentration does not rise. In highly exergonic reactions, the product has little free energy and the opposite reaction requires a large, if not unachievable, amount of energy to reverse the process.



Note that in the above graph, the initial reaction is very steep. As more product forms, the rate of formation levels off.

Coupled Reactions

Exergonic reactions, where free energy is released, can occur spontaneously, but endergonic reactions require an energy input. Cells will often pair a spontaneous exergonic reaction with a nonspontaneous, energy-requiring reaction.

An example of a coupled reaction occurs in the sodium-potassium pump in cell membranes. Energy is released from ATP, the energy-storing molecule. This exergonic action pumps sodium and potassium against the concentration gradient; that is, from an area of low concentration inside the membrane to an area of high concentration outside the membrane, causing an endergonic reaction. Reactions such as these are often seen in cells as the battle to capture and utilize energy occurs.



Coenzymes: chemicals required by a number of enzymes for proper functioning.



DID YOU KNOW?

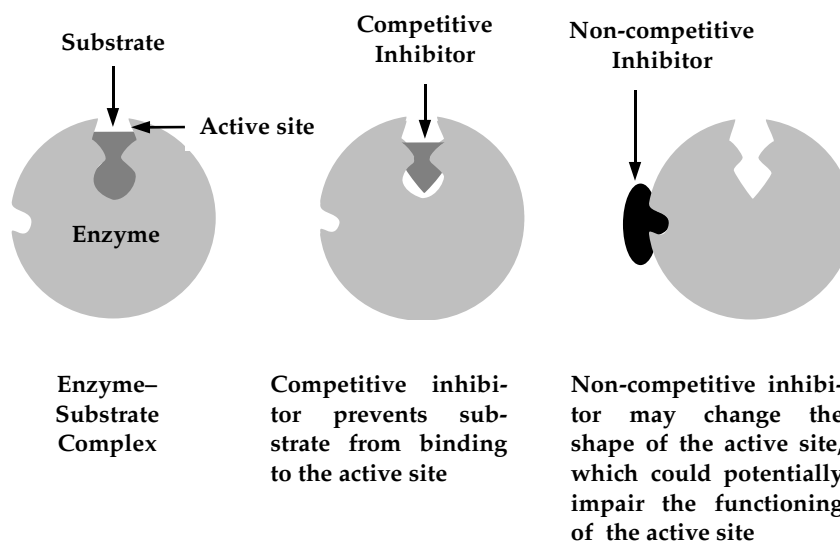
Many toxic substances owe their toxic properties to their ability to act as inhibitors to important enzymes responsible for catalyzing important biological processes. Examples of these toxic substances include cyanide, heavy metals such as lead, mercury, and chromium, and pesticides.

Coenzymes

Enzymes are often tightly bound to a prosthetic group, which is either a metal ion, an organic molecule/metal ion complex, or a small organic molecule called a coenzyme. Metal ions such as Fe^{+3} and Zn^{+2} are generally involved in reactions which require electron removal from a substrate or can electrically bond an enzyme to a substrate.

Coenzymes have a much more varied role. Some are not tightly bound and can move from enzyme to enzyme, transferring electrons or protons. Some alter substrates for a better fit with the enzyme. Still others, bound into membranes, are essential to the energy conversion reactions of photosynthesis and respiration. Many coenzymes must be taken in by animals and are not synthesized; these are collectively referred to as vitamins.

Competitive Inhibitors



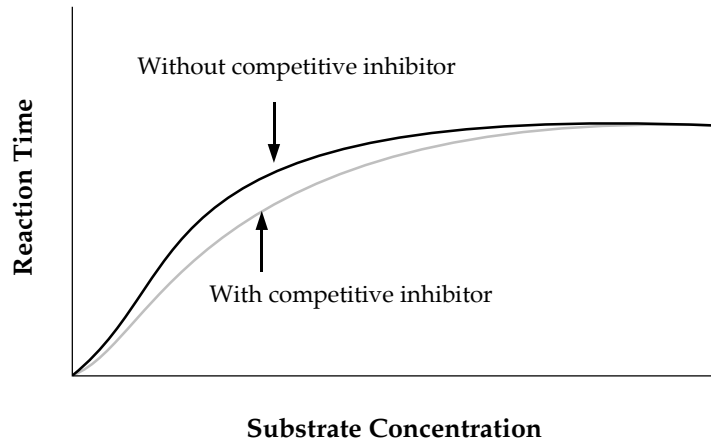
Molecules that bond to the active site and compete with the substrate are called competitive inhibitors. Note that the inhibitor is similar enough to the enzyme that it can fit in the active site, but it is not the same as the substrate. While the bonding of the inhibitor produces no product, the inhibitor blocks the enzyme from bonding to the substrate. Competitive inhibition is reversible and behaves the same as an enzyme-substrate complex, with constant bonding and unbonding of the complex due to the Law of Mass Action. If the concentration of the inhibitor is large enough, the reaction will slow down; otherwise, the inhibitor has little effect.



DID YOU KNOW?

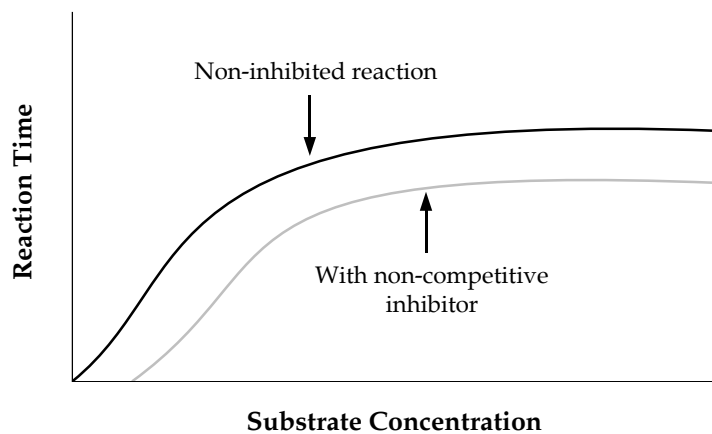
Penicillin, the first of the “wonder drug” antibiotics, permanently blocks the pathways that certain bacteria use to assemble their cell wall components.

The graph below shows the effect of a competitive inhibitor on an enzyme. The reaction without an inhibitor proceeds very rapidly, whereas the reaction that is competitively inhibited proceeds slowly. Eventually, however, the substrate builds up and the competitive inhibitor has no effect.



Noncompetitive Inhibitors

Unlike competitive inhibitors, noncompetitive inhibitors bind to a region of the enzyme other than the active site, causing a shape change in the enzyme that will impair, but not stop, the active site from functioning. Since there is no competition for the active site, the Law of Mass Action will not come into play in this situation, and a buildup of substrate will not make a difference in accelerating the reaction. The graph below shows the noninhibited reaction at a higher rate than the inhibited reaction, despite increasing substrate concentration.





DID YOU KNOW?

Allostery means “different shape”. Allosteric enzymes change shape between active and inactive shapes as a result of the binding of substrates at the active site and/or regulatory molecules at other sites.

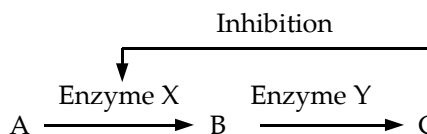


DID YOU KNOW?

Of all known enzymes, catalase is particularly efficient with one of the highest renewal rates — 40 million molecules per second!

Allosteric Controls

More complex enzymes that have quaternary structures contain sites in addition to the active sites which are called effector bonds. The effector actually renders the enzyme inactive only while it is on the enzyme. This is a common mechanism of control in metabolic pathways. In the pathways, enzymes exist in two forms — active and passive. The active form is rendered inactive by an effector, often a product of an earlier enzyme reaction, as illustrated in the diagram below. When the products of a metabolic pathway inhibit an earlier step in the pathway, it is referred to as feedback inhibition, or negative feedback.



Not all allosteric effectors inhibit an enzyme. In allosteric promotion, the effector actually activates an inactive enzyme. Both of these regulatory mechanisms are extremely effective and can actually work together, allowing the cell to store enzymes in both their active and inactive forms. In allosteric inhibition, if a product down the metabolic line begins to build up, it is not to the cell’s advantage to continue to make it. The product itself will “turn down” the reaction until most of the product has been metabolized. In allosteric promotion, that same product (or a different one) will activate an enzyme to begin reaction with a substrate.

The Role of Catalase

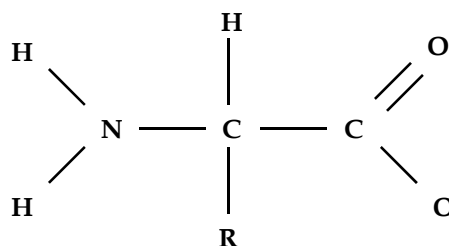
Hydrogen peroxide, an antiseptic that effectively destroys cells, is actually produced in every cell of a human body, formed spontaneously as waste. The enzyme catalase, found in a cell microbody called the peroxisome, breaks down hydrogen peroxide into water and oxygen gas, preventing it from destroying our body’s cells.

Protein Structure

Enzymes are made of long chains of amino acids called polypeptides. Although there are only twenty different amino acids, a polypeptide may have hundreds of amino acids repeated in varying sequenced. All twenty amino acids have an amine group, a carboxyl group, a hydrogen atom, and an R group, as diagrammed below. In aqueous solution, the amine group, NH_2 , ionizes as a base, gaining a proton. The carboxyl group has acidic tendencies, releasing a proton.



Structural proteomics is the field in which scientists attempt to reveal the structure of all the key “functional” sites of any human protein. This will result in the development of highly specific drugs, thus leading to safer and more effective pharmaceuticals.



It is in the structure of the R group that the twenty amino acids differ. Proteins are synthesized on the ribosome; the sequence of amino acids is the primary structure of the protein. Once the polypeptide strand leaves the ribosome, the amino acids begin to interact. The secondary structure involves the formation of helical twists to the protein or, in some cases, a ribbon-like pleated sheet. Once the secondary twists have occurred, the R groups begin to interact, twisting the polypeptide into its final three-dimensional shape. This is called the tertiary structure. In larger enzymes, particularly those showing allostery, a quaternary structure occurs when two or more polypeptides combine.



DID YOU KNOW?

Hydrogen peroxide (H_2O_2) is commonly used for washing out cuts or scrapes on skin. Blood contains catalase, and when the catalase comes in contact with the hydrogen peroxide, it turns the H_2O_2 into water (H_2O) and oxygen gas (O_2). The bubbles and foam that you see are pure oxygen bubbles being created by the catalase.

OBJECTIVES

- Observe the reaction of catalase and hydrogen peroxide
- Demonstrate the effects of extreme temperatures on catalase activity
- Learn how to establish a baseline for the amount of peroxide in a 1.5% solution
- Use titration techniques to determine the rate of hydrogen peroxide decomposition by enzyme catalysis
- Investigate spontaneous decomposition of hydrogen peroxide to oxygen and water

MATERIALS

MATERIALS NEEDED PER GROUP

A. Testing Enzyme Activity

- Hydrogen peroxide, 1.5%, 30 ml
- Catalase working solution, 1 ml
- Boiled catalase working solution, 1 ml
- 1 Syringe, 10 ml
- 1 Pipet, 1 ml
- 1 Glass rod
- 1 Knife or scalpel
- Potato or beef liver
- 2 Plastic cups, 2 oz.
- 1 Beaker, 250 ml

B. Establishing a Baseline — Determining the Amount of Hydrogen Peroxide in a 1.5% Solution

- Hydrogen peroxide, 1.5%, 10 ml
- Catalase working solution, 1 ml
- Sulfuric acid, 1 M, 10 ml
- Potassium permanganate, 2%
- 1 Pipet, 1 ml
- 1 Syringe, 10 ml
- 1 Titration syringe
- 2 Plastic cups, 2 oz.

C. Rate of Hydrogen Peroxide Spontaneous Decomposition

- Hydrogen peroxide, 1.5%, 25 ml
- Catalase working solution, 1 ml
- Sulfuric acid, 1 M, 10 ml
- Potassium permanganate, 2%
- 1 Pipet, 1 ml
- 1 Syringe, 10 ml
- 1 Syringe, 5 ml
- 1 Titration syringe
- 3 Plastic cups, 2 oz.



DID YOU KNOW?

Some enzymes that aid in the digestion of food by humans are:

- Amylase in the salivary glands
- Pepsin in the stomach
- Lipase and trypsin in the small intestine

D. Rate of Hydrogen Peroxide Decomposition by Enzyme Catalysis

Hydrogen peroxide, 1.5%, 60 ml

Catalase working solution, 6 ml

Sulfuric acid, 1M, 60 ml

Potassium permanganate, 2%

- 1 Pipet, 1 ml
- 1 Syringe, 10 ml
- 1 Titration syringe
- 6 Plastic cups
- 1 Stopwatch
- 2 Plastic cups, 2 oz.

PROCEDURE



Wear proper protective equipment such as gloves, safety goggles, and a lab apron.

A. Testing Enzyme Activity

1. Obtain a 10 ml syringe, remove the tip, and label the syringe 'H' for hydrogen peroxide (H_2O_2).
2. Using the syringe, add 10 ml of hydrogen peroxide to a provided 2 oz. plastic cup.
3. Using a pipet, add 1 ml of catalase solution to the cup.
4. Mix the contents by swirling and observe for approximately 30-60 seconds. Record any observations in Table 1 in the Analysis section of the lab.

Effect of Extreme Temperature on Enzyme Activity

1. Using your syringe, dispense 10 ml of hydrogen peroxide solution into a 2 oz. cup.
2. Your instructor has already prepared a sample of boiled catalase for the class. Add 1 ml of the boiled catalase to your cup.
3. Mix the contents by swirling and observe for approximately 30-60 seconds. Record any observations in Table 1.



DID YOU KNOW?

Hydrogen peroxide occurs naturally in rain, snow, atmospheric ozone, and in mountain streams where rushing water is continuously aerated.

Presence of Catalase in Living Tissue



Use extreme caution when working with sharp objects. Never cut an item while holding it in your hand. Cut on a flat surface to avoid causing any bodily harm.

1. Using a knife or scalpel, carefully cut a 1 cm cube of potato or beef liver.
2. Macerate the potato or liver piece with a mortar and pestle. Place the tissue in the 50 ml beaker.



If mortar and pestle are not available, macerate the potato or liver in a 50 ml beaker with a glass rod.

3. Add 10 ml of hydrogen peroxide to the beaker containing the macerated tissue. Observe any reaction that takes place.
4. Record your observations in Table 1. Suggest what might happen if the potato or liver was boiled before it was added to the hydrogen peroxide.

B. Establishing a Baseline — Determining the Amount of Hydrogen Peroxide in a 1.5% Solution



This procedure is written for use with the included titration syringes. The procedure may also be performed with a buret if available.

1. Obtain two more 10 ml syringes, remove the tips, and label one of the syringes 'S' for sulfuric acid. Label the other syringe 'T' for transfer.
2. Dispense 10 ml of hydrogen peroxide into a 2 oz. cup using the properly labeled syringe.
3. Add 1 ml of distilled water to the cup using a pipet.
4. Using the syringe labeled 'S', carefully add 10 ml of sulfuric acid to the cup. Mix the contents by gently swirling.
5. Using the syringe labeled 'T', transfer 10 ml of the mixture into a new 2 oz. plastic cup.
6. Fill a titration syringe to the 10 ml marking with potassium permanganate (KMnO_4). Note the initial reading in Table 2 in the Analysis section of the lab.



DID YOU KNOW?

The enzyme that is produced in most abundance is alpha-amylase. Amylase is the main enzyme in malted barley, which is used in the brewing and distilling industries.

7. Slowly add one drop of potassium permanganate and swirl the solution to mix. Continue to add potassium permanganate, one drop at a time and swirl after each addition, until the solution permanently turns pink or brown. The amount of KMnO_4 added is proportional to the amount of H_2O_2 that was present in the solution.



If you use all of the potassium permanganate in the syringe, refill to the 10 ml mark and continue your titration.

8. Record the final volume in the titration syringe in Table 2.

C. Rate of Hydrogen Peroxide Spontaneous Decomposition

1. Dispense 25 ml of hydrogen peroxide in a 2 oz. plastic cup. Let the beaker sit, uncovered, for 24 hours at room temperature.
2. After 24 hours, dispense 10 ml hydrogen peroxide into a new 2 oz. cup using the properly labeled syringe.
3. Add 1 ml of distilled water to the cup using a pipet.
4. Using the syringe labeled 'S', carefully add 10 ml of sulfuric acid to the cup. Mix the contents by gently swirling.
5. Using the syringe labeled 'T', transfer 10 ml of the mixture into a new 2 oz. plastic cup.
6. Fill a titration syringe to the 10 ml marking with potassium permanganate. Note the initial reading in Table 3 in the Analysis section of the lab.
7. Slowly add one drop of potassium permanganate and swirl the solution to mix. Continue to add potassium permanganate, one drop at a time and swirling after each addition, until the solution permanently turns pink or brown. The amount of KMnO_4 added is proportional to the amount of H_2O_2 that was present in the solution.



If you use all of the potassium permanganate in the syringe, refill to the 10 ml mark and continue your titration.

8. Record the final volume in the titration syringe in Table 3.



DID YOU KNOW?

The main enzyme involved in the ripening of fruit is called pectinase. Pectinase dissolves the pectin “glue” between cells making the whole fruit softer.

D. Rate of Hydrogen Peroxide Decomposition by Enzyme Catalysis

1. Dispense 10 ml of hydrogen peroxide in a 2 oz. plastic cup using the 10 ml syringe labeled 'H'.
2. Using a pipet, add 1 ml of catalase solution and swirl gently for 10 seconds to mix.
3. Using the syringe labeled 'S', add 10 ml of sulfuric acid to stop the reaction.
4. Using the syringe labeled 'T', transfer 10 ml of the mixture into a new 2 oz. plastic cup.
5. Fill a titration syringe to the 10 ml marking with potassium permanganate. Note the initial reading in Table 4 in the Analysis section of the lab.
6. Slowly add one drop of potassium permanganate and swirl the solution to mix. Continue to add potassium permanganate, one drop at a time and swirling after each addition, until the solution permanently turns pink or brown. The amount of KMnO_4 added is proportional to the amount of H_2O_2 that was present in the solution.



If you use all of the potassium permanganate in the syringe, refill to the 10 ml mark and continue your titration.

7. Record the final volume in the titration syringe in Table 4.
8. Repeat the procedure for 30, 60, 120, and 180 seconds.



Remember to reestablish your baseline, as in part B, if more than 24 hours have passed since your last assay.

9. Graph your results in the Analysis section of the lab. Plot the amount of hydrogen peroxide used on the Y-axis and the time, in seconds, on the X-axis. Be sure to properly label your graph.

ANALYSIS

Table 1
Enzyme Activity

Activity	Observations
Enzyme Activity	
Effect of Extreme Temperature	
Presence of Catalase in Living Tissue	

Table 2
Establishing a Baseline

Titration Syringe	OR	Buret
Initial Reading		Initial Reading
Final Reading		Final Reading
Baseline (Initial – Final)		Baseline (Volume used)

Table 3
Hydrogen Peroxide Spontaneous Decomposition Using a Titration Syringe

Initial Reading	
Final Reading	
Volume Used After 24 Hours	
Amount H ₂ O ₂ Spontaneously Decomposed (baseline - volume used after 24 hours)	
% H ₂ O ₂ Spontaneously Decomposed (amount H ₂ O ₂ Spontaneously decomposed/baseline) X 100	

OR

Hydrogen Peroxide Spontaneous Decomposition Using a Buret

Initial Reading	
Final Reading	
Volume Used After 24 Hours	
Amount H ₂ O ₂ Spontaneously Decomposed (baseline - volume used after 24 hours)	
% H ₂ O ₂ Spontaneously Decomposed (amount H ₂ O ₂ Spontaneously decomposed/baseline) X 100	

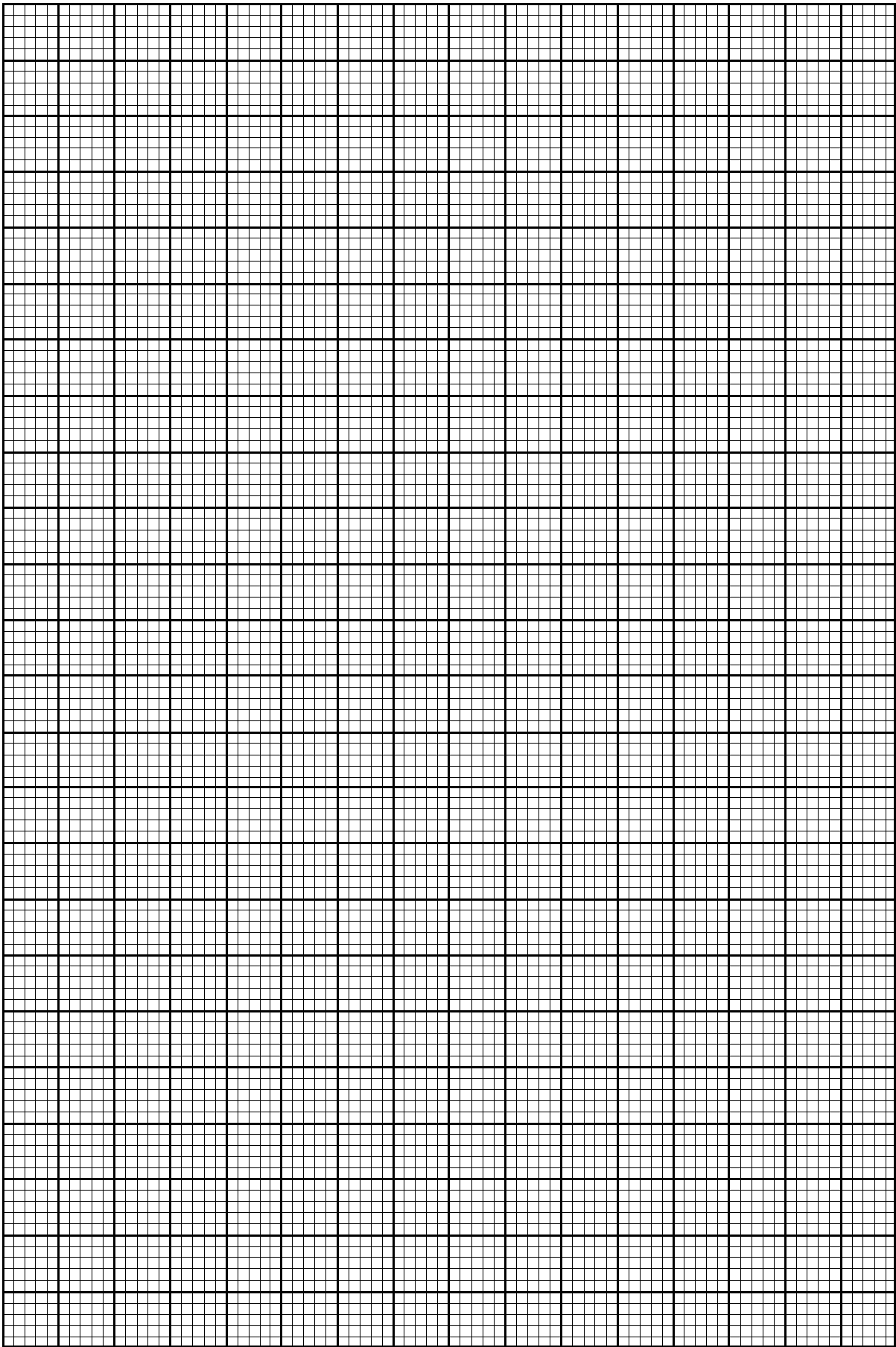
Table 4
Hydrogen Peroxide Decomposition by Enzyme Catalysis
Using a Titration Syringe

	Time (seconds)				
	10	30	60	120	180
Baseline Volume					
Initial Volume					
Final Volume					
Amount KMnO_4 Used (initial – final)					
Amount H_2O_2 Used (baseline – KMnO_4 used)					

OR

Hydrogen Peroxide Decomposition by Enzyme Catalysis
Using a Buret

	Time (seconds)				
	10	30	60	120	180
Baseline Volume					
Initial Volume					
Final Volume					
Amount KMnO_4 Used (initial – final)					
Amount H_2O_2 Used (baseline – KMnO_4 used)					



WARD'S
AP Biology Lab 2
Enzyme Catalysis
Lab Activity

Name: _____
Group: _____
Date: _____

ASSESSMENT

1. What is the function of enzymes in a living system?
2. Different enzymes work better under different conditions. Where in a human body might it be beneficial to have enzymes that work well in very acidic environments?
3. There is a large amount of catalase found in a human liver. Does the liver break down more hydrogen peroxide in the summer or winter? Explain your answer.
4. Many enzymes end with "ase". Come up with your own enzyme, then name and explain what this enzyme does. Draw the enzyme and substrate in the space provided below along with the enzyme-substrate complex.

10. In part D, you investigated the rate of hydrogen peroxide decomposition over time. What purpose did each of the following serve in the experiment?

Catalase –

Hydrogen peroxide –

Sulfuric acid –

Potassium permanganate –

11. Before performing part D of the experiment, why was it necessary to establish a baseline?

12. You may have observed the reaction of naturally-occurring catalase in tissue from either liver or potato. Design an experiment to determine if the amount of catalase varies from tissue to tissue (e.g. 200 g of liver compared to 200 g of potato).

13. Of the thousands of enzymes known, there is a family of enzymes called proteases that catalyze a reaction breaking down proteins. What do you think would happen if you added a protease to your sample of catalase before proceeding with your experiment?