P51 Enzyme Lab

β-Gal Glow™

Contents

At a glance

Measure the effect of pH, temperature, concentration, and competitive inhibition on enzyme reaction rates!

Lab overview

In this activity, a substrate that is not fluorescent under basal conditions will fluoresce brightly when sugar molecules are removed through hydrolysis by the enzyme β-galactosidase. Observing the fluorescence intensity change over time allows students to see the reaction progress and quantify the effect of different variables on enzyme activity.

TECHNIQUES	TOPICS	LEVEL
Micropipetting Fluorescence visualization	Enzymes Biotechnology	General High school Advanced High School College

A blue light illuminator with a tube holder required (see page 5 for details).

Planning your time

- This lab consists of flexible modules designed to be completed in one 90-minute block or two 45-minute classes.
- The lab kit contains enough reagents for eight lab groups to perform at least two of the "Additional investigations".
- You may elect to allow students to choose freely which investigation they will pursue, assign different groups to different investigations, or engage in only one or two of the activities as a class.

Module		Time required
Set up standard curve (required)		~ 10 minutes
Reference reaction (required)		~ 15 minutes
Additional investigations of your choice:		
I.	Substrate and enzyme concentration	~ 40 minutes
II.	Competitive inhibitors	~ 15 minutes
III.	pH	~ 15 minutes
IV.	Temperature	~ 25 minutes

Technical support

If you have any questions about implementing this activity, contact support@minipcr.com

Kit components

Supplied in kit (KT-1900-02)

- Materials are sufficient for eight lab groups.
- All reagents must be stored in the refrigerator.
- Reagents must be used within six months of shipment.

Contents	Provided	Storage
Buffer 1	1,500 µl	Refrigerator
Concentrated Substrate	35 µl	Refrigerator
Dilution Buffer	1,500 µl x 2	Refrigerator
40 µM Fluorescein	1,000 µl	Refrigerator
1X Enzyme	800 µl	Refrigerator
4X Enzyme	100 µl	Refrigerator
100 mM NaOH	700 µl	Refrigerator
500 mM Lactose	500 µl	Refrigerator
0.2 ml plastic tubes	200	Room temp.
1.7 ml plastic tubes	50	Room temp.

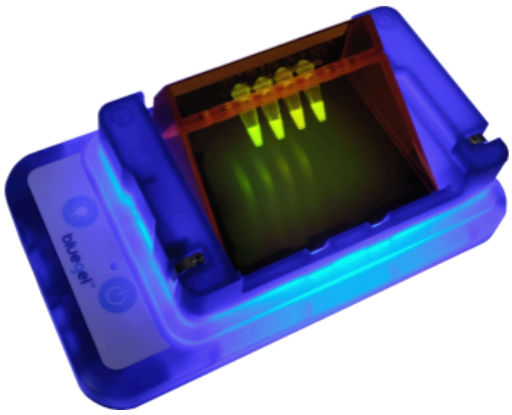
Additional materials needed

Required equipment

available at www.minipcr.com

Item	Recommended quantity
P51 molecular fluorescence viewer or other blue light illuminator with tube holder *e.g.*, blueGel™ paired with a GlowRack™ tube adapter	1 per group
Micropipettes and tips 2-20 µl adjustable 20-200 µl adjustable	 1 pipette per group 1 pipette for teacher prep

P51™ Molecular Fluorescence Viewer **or** blueGel™ paired with a GlowRack™ tube adapter



Other materials supplied by user

- Disposable laboratory gloves
- Protective eyewear
- Fine-tipped permanent marker
- For optional pH investigation: pH paper (suggested)
- For optional temperature investigation: ice, heat block or water bath, thermometer

Teacher prep

- In this activity, students collect data on a reference enzymatic reaction, then observe the effect of different variables on the reaction rate.
- This activity allows students to choose from four investigations:
    I. Substrate and enzyme concentration
    II. Competitive inhibitors
    III. pH
    IV. Temperature
- The lab kit contains enough reagents for eight lab groups to perform at least two of the investigations.
- If dispensing reagents more than a few hours before the lab, store tubes in the refrigerator until use.

Protective gloves and eyewear should be worn for the entirety of this experiment.

Up to one week in advance: prepare and dispense reagents

A. Prepare 2X substrate

1. The Concentrated Substrate may be frozen at 4°C. If this is the case, hold the tube in your hand to thaw.

2. Briefly spin the tube of Concentrated Substrate in a microcentrifuge to ensure all the liquid is collected at the bottom of the tube. If a microcentrifuge is unavailable, you may also shake the liquid to the bottom of the tube with a flick of the wrist.

3. Transfer 30 µl of the Concentrated Substrate to the tube of Buffer 1 and mix thoroughly. This dilutes the substrate to 2X concentration.

4. Relabel the tube of Buffer 1 “2X substrate.”

5. If preparing in advance, store the 2X substrate in the refrigerator. The 2X substrate is stable for two weeks when stored in the refrigerator.

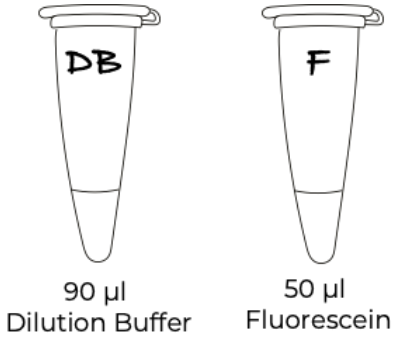
Continued on the next page

B. Dispense regents for the standard curve and reference reaction

- Dispense reagents for student groups in the provided 1.7 ml tubes. (Do not dispense into the 0.2 ml tubes, which students will need to set up their enzymatic assays.)
- If dispensing reagents more than a few hours before the lab, store tubes in the refrigerator until use.

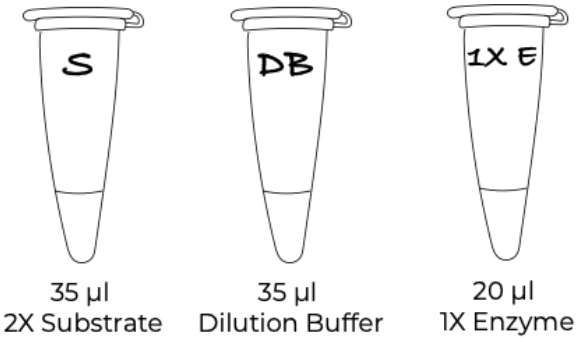
STANDARD CURVE PREPARATION
Every group will need:

Dilution Buffer	90 µl in a 1.7 ml tube
40 µM Fluorescein	50 µl in a 1.7 ml tube
0.2 ml plastic tubes	5



REFERENCE REACTION
Every group will need:

2X substrate	35 µl in a 1.7 ml tube
Dilution Buffer	35 µl in a 1.7 ml tube
1X Enzyme	20 µl in a 1.7 ml tube
0.2 ml plastic tubes	3



Continued on the next page

C. Dispense additional reagents for the investigations of your choice

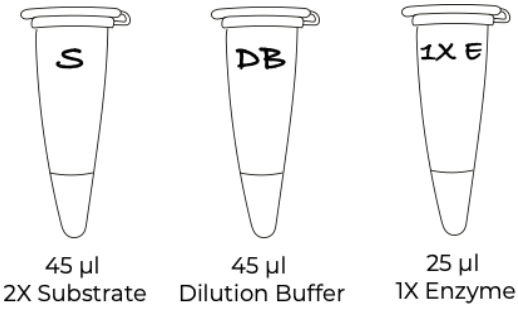
- Dispense reagents for student groups in the provided 1.7 ml tubes. (Do not dispense into the 0.2 ml tubes, which students will need to set up their enzymatic assays.)
- If dispensing reagents more than a few hours before the lab, store tubes in the refrigerator until use.

Investigation I: Substrate and enzyme concentration

We recommend testing the substrate at the following concentrations: 0.25X, 0.5X, 1X, and 2X. The student lab protocol guides students through preparing these samples.

PART A. SUBSTRATE CONCENTRATION
Every group will need:

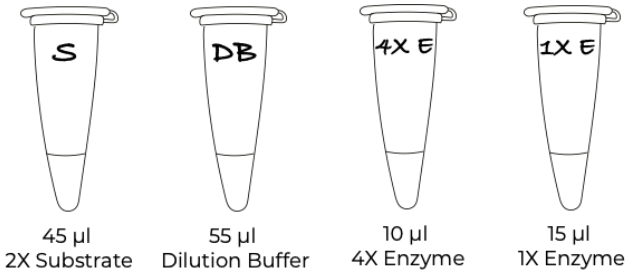
2X substrate	45 µl in a 1.7 ml tube
Dilution Buffer	45 µl in a 1.7 ml tube
1X Enzyme	25 µl in a 1.7 ml tube
0.2 ml plastic tubes	4



We recommend testing the enzyme at the following concentrations: 0.25X, 0.5X, 1X, and 4X. The student lab protocol guides students through preparing these samples.

PART B. ENZYME CONCENTRATION
Every group will need:

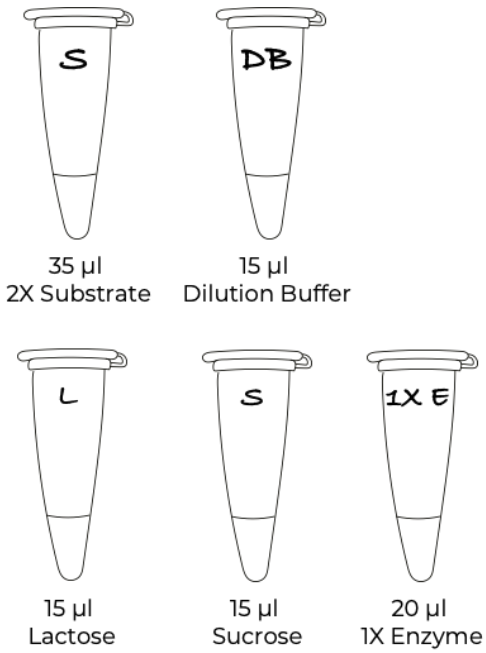
2X substrate	45 µl in a 1.7 ml tube
Dilution Buffer	55 µl in a 1.7 ml tube
4X Enzyme	10 µl in a 1.7 ml tube
1X Enzyme	15 µl in a 1.7 ml tube
0.2 ml plastic tubes	6



Continued on the next page

Investigation II: Competitive inhibitors

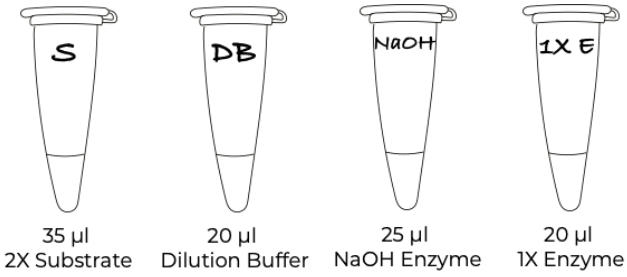
Every group will need:	
2X substrate	35 µl in a 1.7 ml tube
Dilution Buffer	15 µl in a 1.7 ml tube
Lactose	15 µl in a 1.7 ml tube
Sucrose	15 µl in a 1.7 ml tube
1X Enzyme	20 µl in a 1.7 ml tube
0.2 ml plastic tubes	3



Investigation III: pH

We recommend testing at approximately the following pH levels: pH ~ 6 pH ~ 7, and pH ~ 9. The student lab protocol guides students through preparing these samples.

Every group will need:	
2X substrate	35 µl in a 1.7 ml tube
Dilution Buffer	20 µl in a 1.7 ml tube
NaOH	25 µl in a 1.7 ml tube
1X Enzyme	20 µl in a 1.7 ml tube
0.2 ml plastic tubes	3



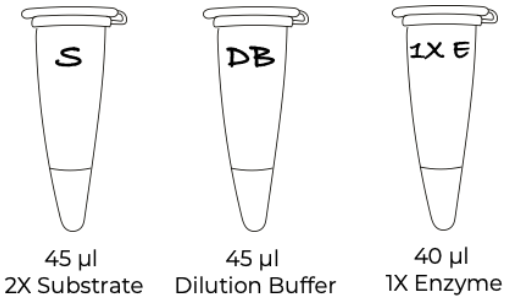
Additional materials provided by user:

- pH paper (optional)

Investigation IV: Temperature

We recommend testing the following conditions: ice bath, room temperature, 55 °C, and 75 °C or greater.

Every group will need:	
2X substrate	45 µl in a 1.7 ml tube
Dilution Buffer	45 µl in a 1.7 ml tube
1X Enzyme	40 µl in a 1.7 ml tube
0.2 ml plastic tubes	8



Additional materials provided by user:

- Heat source (water bath or heat block)
- Ice
- Thermometer (optional)

The day of the lab: Distribute supplies and reagents to lab groups

At the start of this experiment, every lab group should have:	
Materials to prepare the standard curve	Refer to page 7 for detailed list
Materials to collect reference reaction data	Refer to page 7 for detailed list
Materials for the selected additional investigations	
Investigation I: Substrate and enzyme concentration	Refer to page 8 for detailed list
Investigation II: Competitive inhibitors	Refer to page 9 for detailed list
Investigation III: pH	Refer to page 9 for detailed list
Investigation IV: Temperature	Refer to page 10 for detailed list
2-20 µl micropipette and tips	
P51 Molecular Fluorescence Viewer with the yellow filter* in place	

*The P51 comes with a yellow filter and an orange filter. While either filter will work to visualize the fluorescent reaction product in this activity, the yellow filter gives better results.

Student guide

Background information

Enzymes

When we think of life, we think of dynamic processes: change, growth, movement. For many of these processes the most central molecules are enzymes — proteins that catalyze (speed up) chemical reactions. Enzymes work in pathways to break down or build up molecules, they digest your food, they generate the movement of the body, they transport molecules in and out of cells, they replicate and transcribe your DNA. If you can think of a process or an action that is actively carried out in the cell, an enzyme is probably central to it. In fact, the primary role of DNA is to carry the instructions for making proteins, chief among them enzymes. Without these molecules, life as we know it would not exist.

Enzymes are proteins, and like all proteins, enzymes are polymers made of amino acid monomers that are folded into precise three-dimensional shapes. Enzymes work by binding very specifically to a certain molecule or molecules, called the *substrate*. The site on the enzyme where the substrate binds is known as the *active site*. When bound to an active site, the substrate is oriented in such a way that a chemical reaction is more likely to occur. As catalysts, enzymes speed up chemical reactions by lowering the amount of energy needed for the reaction to start. Enzymes don't make reactions happen that otherwise wouldn't; they just speed them up—often significantly. Some enzymes can speed to mere milliseconds reactions that may otherwise take millions of years.

In an enzyme catalyzed reaction, substrates are changed by the reaction, but the enzyme is not. This means that the same enzyme can catalyze a reaction repeatedly as long as there is substrate for it to react with. Substrate, however, is permanently changed in the reaction.

In an enzyme catalyzed reaction, the substrate (orange) fits into the active site of the enzyme (blue) due to the unique shape and chemical properties of the active site. The substrate is changed by the reaction, but the enzyme is not.

Enzymes typically work best in the very specific physiological environments where they carry out their biological roles. This is because evolution has shaped each type of enzyme to function optimally in the conditions under which they are typically found, and enzymes tend to be highly susceptible to changes in these conditions. Factors such as pH, temperature, concentration, and other molecules present in solution can have profound effects on an enzyme's efficiency. The effect of each is discussed below.

Enzyme and substrate concentration

Individual enzymes catalyze single reactions at a time, so how quickly a reaction progresses can depend heavily on both the amount of enzyme and substrate in solution.

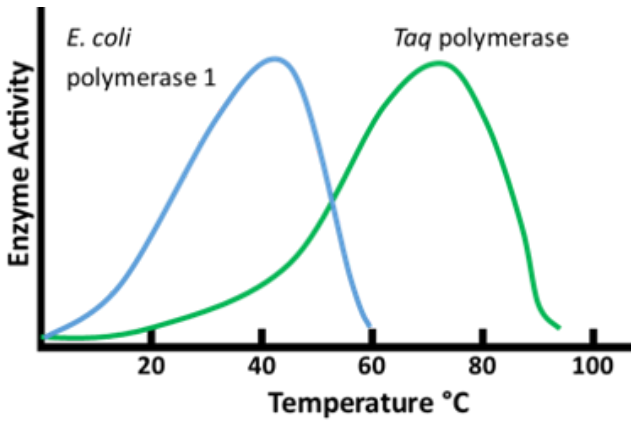
Enzymes and substrates interact when they come in contact with each other in the correct conformation. But enzymes and substrates typically only come in contact through random movement of molecules in solution, so-called Brownian motion. The more molecules of an enzyme that are present in solution, the more frequently an enzyme and substrate will come in contact and react. The fewer molecules of enzyme in solution, the less often that will happen. For this reason, as a rule, for a given amount of substrate, adding more molecules of enzyme in solution will cause a reaction to proceed more quickly. Fewer molecules of enzyme in solution lead to a reaction proceeding more slowly.

The same is true for substrates. As more substrate is added to solution enzymes will encounter the substrates at a greater rate, increasing the rate of the reaction. Adding enough substrate, however, will result in enzymes becoming saturated. This occurs when there is so much substrate that all available enzyme molecules in solution are bound to a substrate, and as soon as an enzyme and substrate react, a new molecule of substrate binds to the enzyme. Under these conditions adding more substrate will not increase the reaction rate. Lowering substrate concentration below the saturation point will reduce the speed of the reaction, as at lower concentrations enzyme and substrate will again be less likely to meet in solution.

When a reaction is proceeding, the concentration of substrate will decrease over time due to it being processed by the enzyme. Therefore, the rate of an enzymatic reaction is expected to slow as it progresses. For this reason, when measuring reaction rates scientists usually measure the initial reaction rate, the rate of the reaction when enzyme and substrate are first combined.

Temperature

Most enzymes function best in a relatively narrow temperature range. As a general rule, when a system is too cold, the lack of energy in the system will slow reactions down. As more heat is added to a system, the general increase in energy will cause reactions to occur more quickly. Adding too much heat to the system, however, can destabilize and change the shape of the enzyme. If an enzyme is too hot for too long, it may permanently

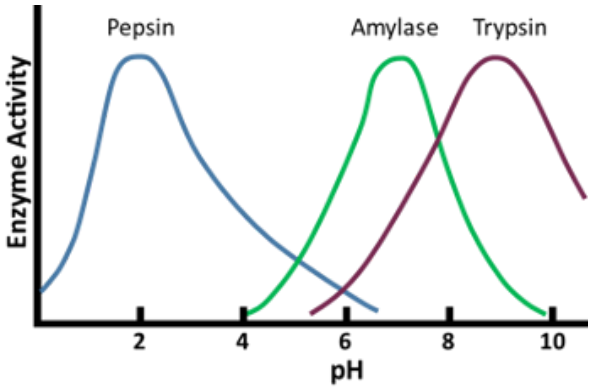


Approximate relative activity of two different polymerase enzymes at different temperatures.

change shape or *denature*, breaking its three-dimensional conformation thus permanently impairing its ability to catalyze reactions. Depending on the organism that the enzyme normally operates in, the amount of heat energy needed to denature an enzyme can vary greatly. For example, *E. coli* DNA polymerase 1 and *Thermus aquaticus* (*Taq*) DNA polymerase are both DNA polymerases that are found in bacteria. *E. coli* generally live in the guts of other animals, and for this reason, *E. coli* enzymes tend to work well at around 37-40° C, about body temperature. *Thermus aquaticus* is a thermophilic organism that lives in hot springs, and therefore *Taq* polymerase functions best at temperatures above 70° C—hot enough to burn you.

pH

pH is a measure of the relative amount of positively charged hydrogen (H^+) ions and negatively charged hydroxide (OH^-) ions in solution. Changes in the concentration of these two ions can influence enzyme catalyzed reactions in two ways.



Approximate relative activity of three different enzymes at different pH values.

First, the active site of an enzyme contains amino acids that will form temporary bonds with the substrate. An increase or decrease in H^+ ions in solution can interfere with the ionization state of the amino acids in the active site, altering their charge. Such a change will greatly affect the ability of the substrate to bind within the active site, reducing or eliminating the ability for the enzyme to catalyze the reaction.

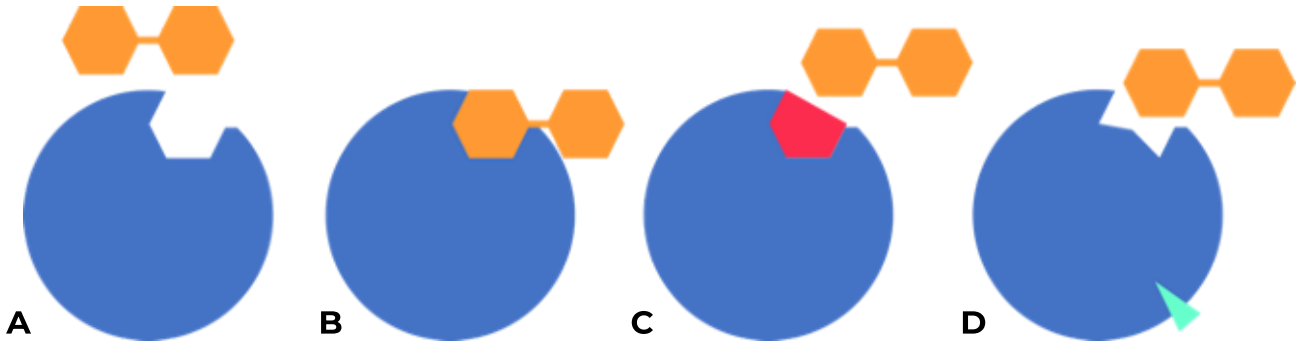
Second, the specific three-dimensional structure of an enzyme must be maintained for the enzyme to function properly. This structure relies on hydrogen bonds between amino acids to maintain the stability. Hydrogen bonds are relatively weak interactions between polar amino acids. Charged H^+ or OH^- ions can bind to the polar regions of amino acids, interfering with their ability to bond with other regions of the enzyme. Affecting hydrogen bonds in this way can result in the three-dimensional structure of the enzyme becoming unstable and losing its catalytic ability. Changing pH by too much can lead to the enzyme denaturing, permanently altering its shape and ability as a catalyst.

Like temperature, though, different enzymes have evolved to function optimally in different pH conditions. Pepsin, for example, is an enzyme that breaks the peptide bonds of proteins. Pepsin is found in your stomach, and so is optimized to function at extremely low (acidic) pH values (high H^+ concentration). In contrast, amylase is an enzyme produced in your salivary glands and pancreas and operates best at more typical neutral pH values found in the body (close to pH 7), breaking down starches into simple sugars. Trypsin, another enzyme that breaks down proteins, is active in the small intestine and functions best at a pH greater than 8.

Inhibition

Molecules that can interfere with enzymatic activity are called inhibitors. Competitive inhibitors work by occupying the active site of the enzyme, physically blocking the substrate from binding. Non-competitive inhibitors bind to a region of the enzyme that is separate from the active site, usually changing the conformation of the enzyme slightly, thereby impairing the enzyme's ability to catalyze the reaction. Because competitive inhibitors physically block the active site, but do not change the structure of the enzyme, they can slow reactions, but generally do not stop the enzyme from catalyzing the reaction altogether. And if a substrate is in a much higher concentration than a competitive inhibitor, the inhibitor will have little effect because the active site is more likely to encounter substrate than inhibitor. If the inhibitor is in higher concentration than the substrate, it will have more of an effect, because the enzyme is more likely to come in contact with and bind to the inhibitor than it is to the substrate.

Non-competitive inhibitors are not affected by the concentration of substrate. Because the substrate and inhibitor bind to different regions of the enzyme, changing substrate concentration does not affect the inhibitor binding to the enzyme. With a non-competitive inhibitor, even with very large concentrations of substrate, the reaction will still be inhibited.

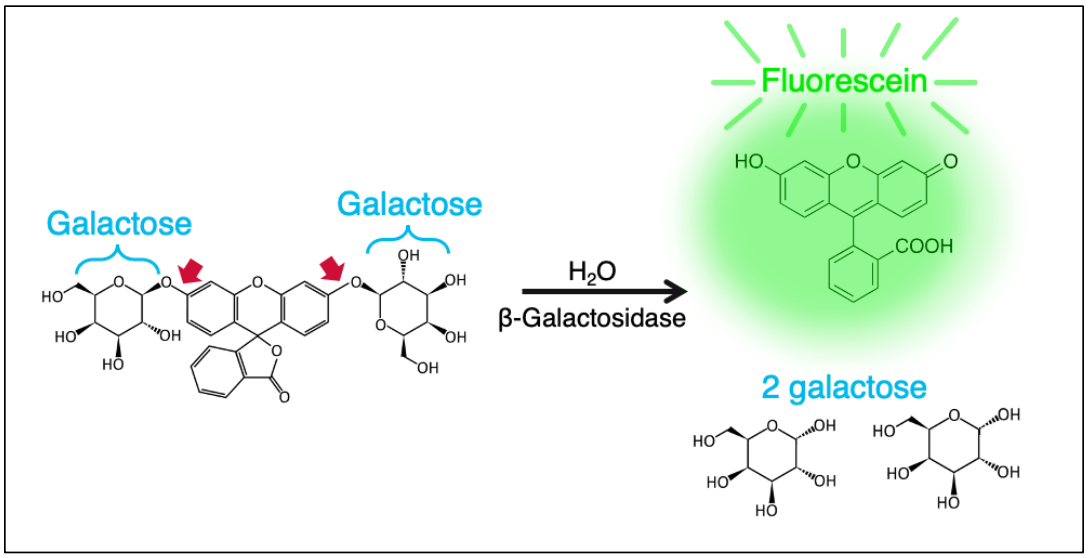


In a typical reaction (B), the substrate fits in the active site of the enzyme because of the unique shape and chemical properties of the active site. In the presence of a competitive inhibitor (C), another molecule binds to the active site, physically blocking the substrate from binding. In non-competitive inhibition (D), a molecule binds to a location on the enzyme separate from the active site, changing the conformation of the active site.

Today's lab

You will be investigating the function of β-galactosidase. β-galactosidase is a type of enzyme that is found in many organisms, from bacteria to humans. It catalyzes the hydrolysis of the glycosidic bond between the monosaccharide galactose and other carbohydrates. It is a specific form of β-galactosidase, lactase, that gives mammals the ability to digest milk. Inside you, lactase breaks the glycosidic bond in the primary sugar found in milk, lactose. Lactose consists of a galactose and a glucose monomer, linked to form a disaccharide. β-galactosidase will break that bond, but it will also readily break any glycosidic bond between galactose and another molecule. This is because the active site of the β-galactosidase enzyme only binds to the galactose portion of the substrate and is not dependent on the structure of other components of the molecule.

In this lab, you will be using the enzyme β-galactosidase to catalyze the breakdown of a molecule that contains two galactose monomers bound to a fluorescein molecule. Fluorescein is a molecule that will appear bright fluorescent green when exposed to blue light and is commonly used in biological applications. But fluorescein will not fluoresce when bound to the two galactose monomers in the substrate; only when the galactose monomers are no longer bound to the fluorescein will fluorescence be observed.



To measure the progression of your reactions, you will be comparing the brightness of fluorescence in your reaction as a measure of how much fluorescein has been produced. To quantify brightness, you will first create a standard curve using known fluorescein concentrations. By comparing the brightness in your reaction to a standard curve of fluorescein, you will be able to measure the relative progression of the reaction over time and obtain an estimate of the reaction rate.

Enzyme Lab: β-Gal Glow lab protocol

Protective gloves and eyewear should be worn for the entirety of this experiment.

Set up standard curve

Goal: You will create a standard curve of fluorescein to use as a reference for comparing your enzymatic reactions.

Materials needed for this section:

- Dilution Buffer
- Fluorescein
- Five empty 0.2 ml tubes
- P51 Molecular Fluorescence Viewer
- Micropipette and tips (2-20 µl range)
- Fine-tipped permanent marker

Procedure:

1. Label five 0.2 ml tubes 1-5.
2. Add reagents to the tubes according to the table below:

Standard tube	1	2	3	4	5
Dilution Buffer	20 µl	20 µl	20 µl	20 µl	-
40 µM Fluorescein	-	-	-	20 µl	20 µl

3. Remove 20 µl from tube 4 and transfer it to tube 3. Mix well by pipetting up and down.
4. Remove 20 µl from tube 3 and transfer it to tube 2. Mix well by pipetting up and down.
5. Remove 20 µl from tube 2 and transfer it to tube 1. Mix well by pipetting up and down.
6. Remove 20 µl from tube 1 and discard.
7. Visually check that all five tubes have approximately the same volume. Each tube should contain 20 µl fluorescein solution.
8. Place tubes 1 through 5 in the P51 Molecular Fluorescence Viewer with tube 1 on the left as you look at the front of the viewer. This is your standard curve.
9. Turn on the blue light and observe the tubes through the yellow window. The brightness of the fluorescence should increase in every tube from tubes 1 to 5. If this is not the case, return to step 1 on this page and prepare a new set of standard curve samples.
10. Turn off the blue light, but leave the standard curve samples in the P51 Molecular Fluorescence Viewer. You will use them throughout the experiment.

    Note: Try to limit the exposure of the standard curve samples to the blue light to prevent photobleaching.

Choose team role

You will observe each enzymatic reaction for two minutes, collecting data every 15 seconds by estimating the brightness in the enzymatic reaction tube compared to the standard curve. We recommend assigning different members of your group the following roles:

- **Timekeeper**: Keep track of time and state when data should be collected.
- **Observer**: Watch the experimental reaction and assign a brightness score every 15 seconds.
- **Data recorder**: Enter the data into a data table. If there are only two people in your group, the data recorder can also perform the observer role.

Collect reference reaction data

Goal: In this section, you will observe the reference enzymatic reaction. You will perform three trials to familiarize yourself with the experimental procedure and data collection.

Materials needed for this investigation:

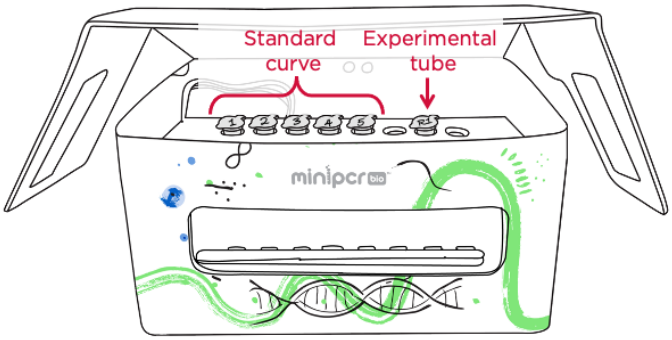
- 2X substrate
- Dilution Buffer
- 1X Enzyme
- Three empty 0.2 ml tubes
- P51 Molecular Fluorescence Viewer
- Micropipette and tips (2-20 µl range)
- Timer
- Fine-tipped permanent marker

Procedure:

1. Label three 0.2 ml tubes R1, R2, and R3.
2. Prepare the substrate by adding reagents to the tubes according to the table below.

Experimental condition	Reference reaction Trial 1	Reference reaction Trial 2	Reference reaction Trial 3
Tube label	R1	R2	R3
2X Substrate	10 µl	10 µl	10 µl
Dilution Buffer	10 µl	10 µl	10 µl

3. Place tube R1 in position 7 of the P51 Molecular Fluorescence Viewer, adjacent to the standard curve. Leave the P51's blue light off.



4. Please read through steps 5-8 below so you understand what to do in the next section of this protocol.
5. Turn on the P51's blue light, and leave it on.
6. Keeping tube R1 in the P51 viewer, open the tube and add 5 µl of 1X Enzyme directly into the liquid. Mix by slowly pipetting up and down three times.
7. As soon as the enzyme is added, each member should follow the instructions below for their assigned role:

   Timekeeper
   a. Start a stopwatch.
   b. Every 15 seconds, tell the person in your group acting as the observer to collect data. Your team needs to collect data at the following time points (in min:sec):

0:15	0:30	0:45	1:00	1:15	1:30	1:45	2:00

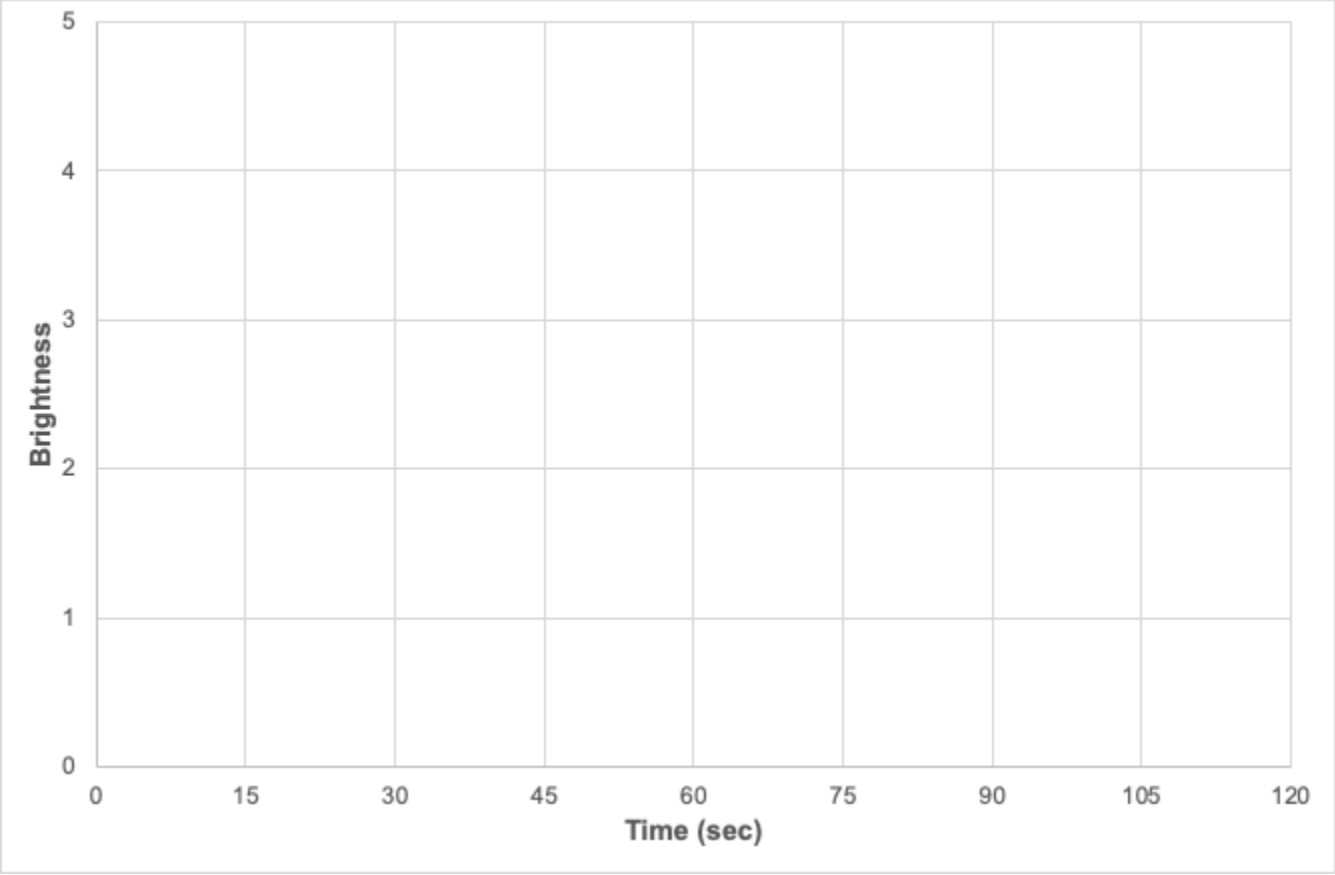
   Observer
   a. When the timekeeper tells you to collect data, estimate the brightness in the enzymatic reaction tube by comparing it to the five samples in the standard curve (1-5). Assign a brightness score (1 to 5) based on the closest match.
   b. Share the score out loud with the data recorder so they can enter it into the spreadsheet.

   Data recorder
   a. If you have someone in your group dedicated to being the data recorder, we recommend entering the data directly into a spreadsheet in a program like Google Sheets or Microsoft Excel. You can find a template here.
   b. If the same person is observing the reactions and recording the data, it might be easier to write down the brightness scores in the table provided on the next page. The times have been converted into seconds to facilitate graphical analysis.
8. After collecting data for 2 minutes, remove tube R1 from the P51 Molecular Fluorescence Viewer. You can discard the tube as instructed by your teacher.
9. Repeat these steps to collect data for tube R2:
   a. Place tube R2 in position 7 of the P51, adjacent to the standard curve.
   b. Keeping tube R2 in the P51 viewer, open the tube and add 5 µl of 1X Enzyme directly into the liquid. Mix by slowly pipetting up and down three times.
   c. Collect data every 15 seconds for 2 minutes.
   d. Remove tube R2 from the P51 Molecular Fluorescence Viewer.
10. Repeat these steps to collect data for tube R3:
    a. Place tube R3 in position 7 of the P51, adjacent to the standard curve.
    b. Keeping tube R3 in the P51 viewer, open the tube and add 5 µl of 1X Enzyme directly into the liquid. Mix by slowly pipetting up and down three times.
    c. Collect data every 15 seconds for 2 minutes.
    d. Remove tube R3 from the P51 Molecular Fluorescence Viewer.
11. Turn off the P51's blue light.

Reference reaction									
Time (sec)	0	15	30	45	60	75	90	105	120
Trail 1 (Tube R1)	0								
Trial 2 (Tube R2)	0								
Trail 3 (Tube R3)	0								

Data analysis for reference reaction

Graph the average (mean) of the results. The empty graph below illustrates how the graph should be set up. The X-axis should represent time, and the Y-axis should represent the brightness of the reaction as compared to your standard curve.



Proceed to the additional investigations assigned by your teacher

Investigation I: Substrate and enzyme concentration

Part A. Substrate concentration

Research question	What is the relationship between substrate concentration and the enzymatic reaction rate?
Experimental plan	You will collect data for four substrate concentrations: 0.25X, 0.5X, 1X, and 2X
State your hypothesis	
Justify your hypothesis	

Materials needed for this investigation:

- 2X substrate
- Dilution Buffer
- 1X Enzyme
- Four empty 0.2 ml tubes
- P51 Molecular Fluorescence Viewer
- Micropipette and tips (2-20 µl range)
- Timer
- Fine-tipped permanent marker

Procedure:

1. Label four 0.2 ml tubes SA, SB, SC, and SD.
2. Add reagents to the tubes according to the table below.

Experimental condition	0.25X substrate	0.5X substrate	1X substrate	2X substrate
Tube label	SA	SB	SC	SD
2X Substrate	2.5 µl	5 µl	10 µl	20 µl
Dilution Buffer	17.5 µl	15 µl	10 µl	-

3. Place tube SA in position 7 of the P51 Molecular Fluorescence Viewer, adjacent to the standard curve. Leave the P51's blue light off.
4. Read through steps 5-8 below so you are prepared for what needs to be done in the next section of this protocol.
5. Turn on the P51's blue light, and leave it on.
6. Keeping tube SA in the P51 viewer, open the tube and add 5 µl of 1X Enzyme directly into the liquid. Mix by slowly pipetting up and down three times.

7. As soon as the enzyme is added, each member should follow the instructions below for their assigned role:

   Timekeeper

   a. Start a stopwatch.
   b. Every 15 seconds, tell the person in your group acting as the observer to collect data. Your team needs to collect data at the following time points (min:sec):

0:15	0:30	0:45	1:00	1:15	1:30	1:45	2:00

   Observer

   a. When the timekeeper tells you to collect data, estimate the brightness in the enzymatic reaction tube by comparing it to the five samples in the standard curve. Assign a brightness score (1 to 5) based on the closest match.
   b. Share the score out loud with the data recorder so they can enter it into the spreadsheet.

   Data recorder

   a. If you have someone in your group dedicated to being the data recorder, we recommend entering the data directly into a spreadsheet in a program like Google Sheets or Microsoft Excel. You can find a template here.
   b. If the same person is observing the reactions and recording the data, it might be easier to write the brightness scores down in the table provided on the next page. The times have been converted into seconds to facilitate graphical analysis.

8. After collecting data for 2 minutes, remove tube SA from the P51 Molecular Fluorescence Viewer. You can discard the tube as instructed by your teacher.

9. Repeat these steps to collect data for tube SB:
   a. Place tube SB in position 7 of the P51, adjacent to the standard curve.
   b. Keeping tube SB in the P51 viewer, open the tube and add 5 µl of 1X Enzyme directly into the tube. Mix by slowly pipetting up and down three times.
   c. Collect data every 15 seconds for 2 minutes.
   d. Remove tube SB from the P51 Molecular Fluorescence Viewer.

10. Repeat these steps to collect data for tube SC:
    a. Place tube SC in position 7 of the P51, adjacent to the standard curve.
    b. Keeping tube SC in the P51 viewer, open the tube and add 5 µl of 1X Enzyme directly into the tube. Mix by slowly pipetting up and down three times.
    c. Collect data every 15 seconds for 2 minutes.
    d. Remove tube SC from the P51 Molecular Fluorescence Viewer.

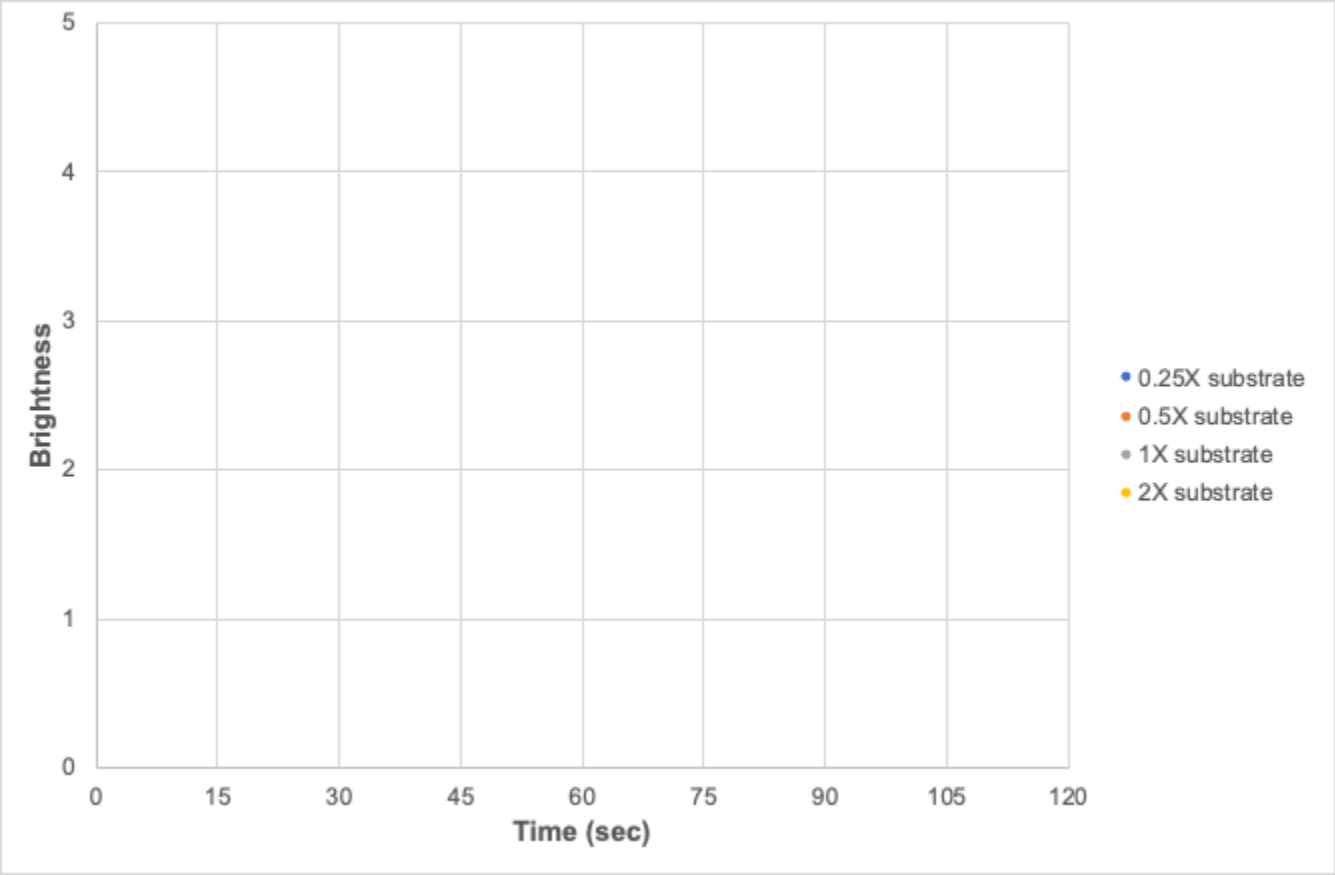
11. Repeat these steps to collect data for tube SD:
    a. Place tube SD in position 7 of the P51, adjacent to the standard curve.
    b. Keeping tube SD in the P51 viewer, open the tube and add 5 µl of 1X Enzyme directly into the tube. Mix by slowly pipetting up and down three times.
    c. Collect data every 15 seconds for 2 minutes.
    d. Remove tube SD from the P51 Molecular Fluorescence Viewer.

12. Turn off the P51's blue light.

Substrate concentration investigation									
Time (sec)	0	15	30	45	60	75	90	105	120
0.25X substrate (Tube SA)	0								
0.5X substrate (Tube SB)	0								
1X substrate (Tube SC)	0								
2X substrate (Tube SD)	0								

Data analysis for Investigation I.A: Substrate concentration

1. Graph the results. The empty graph below illustrates how the graph should be set up. The X-axis should represent time, and the Y-axis should represent the brightness of the reaction as compared to your standard curve. A legend with the different experimental conditions should also be included.



2. Use the information in the graph to complete the CER table on the next page.

CER Table for Investigation I.A: Substrate concentration

Fill in the table based on your lab results. Refer to the CER rubric on page 27.

Question:
What is the relationship between substrate concentration and the enzymatic reaction rate?

Claim Make a clear statement that answers the above question	
Evidence Provide data from the lab that supports your claim	
Reasoning Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.	

Score	4	3	2	1
CLAIM A statement that answers the original question/problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is hightly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric Score	3	4	5	6	7	8	9	10	11	12
Equivalent	55	60	65	70	75	80	85	90	95	100

Part B. Enzyme concentration

Research question	What is the relationship between enzyme concentration and the enzymatic reaction rate?
Experimental plan	You will collect data for four enzyme concentrations: 0.25X, 0.5X, 1X, and 4X
State your hypothesis	
Justify your hypothesis	

Materials needed for this investigation:

- 2X Substrate
- Dilution Buffer
- 4X Enzyme
- 1X Enzyme
- Six empty 0.2 ml tubes
- P51 Molecular Fluorescence Viewer
- Micropipette and tips (2-20 µl range)
- Timer
- Fine-tipped permanent marker

Procedure:

1. Label four 0.2 ml tubes EA, EB, EC, and ED.
2. Prepare the substrate by adding reagents to the tubes according to the table below.

Experimental condition	0.25X enzyme	0.5X enzyme	1X enzyme	4X enzyme
Tube label	EA	EB	EC	ED
2X Substrate	10 µl	10 µl	10 µl	10 µl
Dilution Buffer	10 µl	10 µl	10 µl	10 µl

3. Label two 0.2 ml tubes 0.25X and 0.5X
4. Prepare the 0.25X and 0.5X enzyme dilutions by adding reagents to the tubes according to the table below.

Tube label	0.25X	0.5X
Dilution Buffer	7.5 µl	5 µl
1X enzyme	2.5 µl	5 µl

5. Place tube EA in position 7 of the P51 Molecular Fluorescence Viewer, adjacent to the standard curve. Leave the P51's blue light off.

6. Read through steps 7-10 below so you are prepared for what needs to be done in the next section of this protocol.
7. Turn on the P51's blue light, and leave it on.
8. Keeping tube EA in the P51 viewer, open the tube and add 5 µl of 0.25X enzyme that you prepared, directly into the liquid. Mix by slowly pipetting up and down three times.
9. As soon as the enzyme is added, each member should follow the instructions for their assigned role, as described below:

   Timekeeper
   a. Start a stopwatch.
   b. Every 15 seconds, tell the person in your group acting as the observer to collect data. Your team needs to collect data at the following time points (min:sec):

0:15	0:30	0:45	1:00	1:15	1:30	1:45	2:00

   Observer
   a. When the timekeeper tells you to collect data, estimate the brightness in the enzymatic reaction tube by comparing it to the five samples in the standard curve. Assign a brightness score (1 to 5) based on the closest match.
   b. Share the score out loud with the data recorder so they can enter it into the spreadsheet.

   Data recorder
   a. If you have someone in your group dedicated to being the data recorder, we recommend entering the data directly into a spreadsheet in a program like Google Sheets or Microsoft Excel. You can find a template here.
   b. If the same person is observing the reactions and recording the data, it might be easier to write the brightness scores down in the table provided on the next page. The times have been converted into seconds to facilitate graphical analysis.

10. After collecting data for 2 minutes, remove tube EA from the P51 Molecular Fluorescence Viewer. You can discard the tube as instructed by your teacher.
11. Repeat these steps to collect data for 0.5X enzyme:
    a. Place tube EB in position 7 of the P51, adjacent to the standard curve.
    b. Keeping tube EB in the P51 viewer, open the tube and add 5 µl of 0.5X enzyme that you prepared, directly into the liquid. Mix by slowly pipetting up and down three times.
    c. Collect data every 15 seconds for 2 minutes.
    d. Remove tube EB from the P51 Molecular Fluorescence Viewer.
12. Repeat these steps to collect data for 1X enzyme:
    a. Place tube EC in position 7 of the P51, adjacent to the standard curve.
    b. Keeping tube EC in the P51 viewer, open the tube and add 5 µl of 1X enzyme directly into the liquid. Mix by slowly pipetting up and down three times.
    c. Collect data every 15 seconds for 2 minutes.
    d. Remove tube EC from the P51 Molecular Fluorescence Viewer.

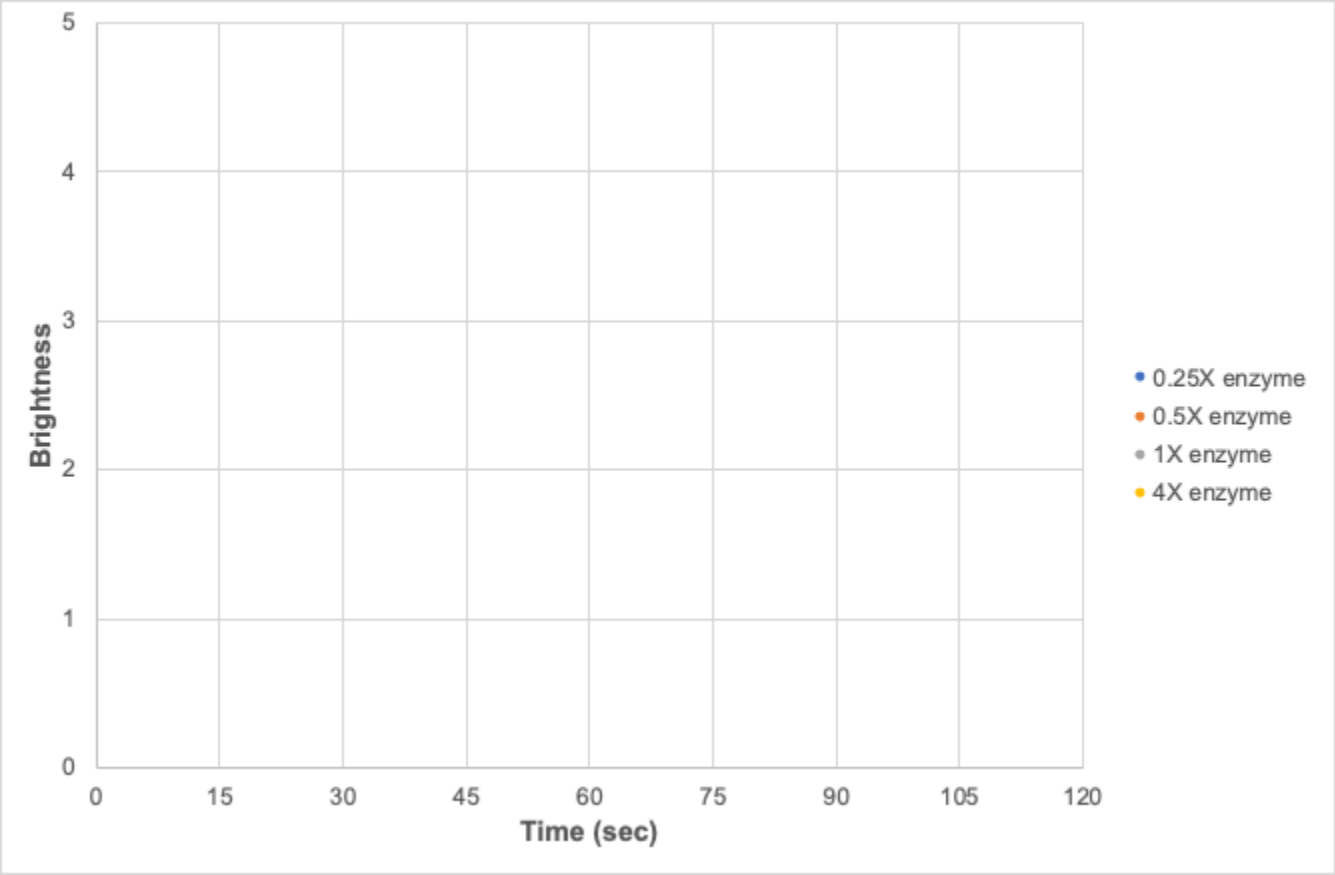
13. Repeat these steps to collect data for 4X enzyme:
    a. Place tube ED in position 7 of the P51, adjacent to the standard curve.
    b. Keeping tube ED in the P51 viewer, open the tube and add 5 µl of 4X enzyme directly into the liquid. Mix by slowly pipetting up and down three times.
    c. Collect data every 15 seconds for 2 minutes.
    d. Remove tube ED from the P51 Molecular Fluorescence Viewer.

14. Turn off the P51's blue light.

Enzyme concentration investigation									
Time (sec)	0	15	30	45	60	75	90	105	120
0.25X enzyme (Tube EA)	0								
0.5X enzyme (Tube EB)	0								
1X enzyme (Tube EC)	0								
4X enzyme (Tube ED)	0								

Data analysis for Investigation I.B: Enzyme concentration

1. Graph the results. The empty graph below illustrates how the graph should be set up. The X-axis should represent time, and the Y-axis should represent the brightness of the reaction as compared to your standard curve. A legend with the different experimental conditions should also be included.



2. Use the information in the graph to complete the CER table on the next page.

3. Critical thinking: Which variable seemed to have a greater effect on the reaction rate, changing the substrate concentration or the enzyme concentration? Explain what might be the reason for this.

4. Critical thinking: Which seemed to have a greater effect on the total amount of product, changing the substrate concentration or changing the enzyme concentration? Explain what might be the reason for this.

CER Table for Investigation I.B: Enzyme concentration

Fill in the table based on your lab results. Refer to the CER rubric on page 27.

Question:
What is the relationship between enzyme concentration and the enzymatic reaction rate?

Claim Make a clear statement that answers the above question	
Evidence Provide data from the lab that supports your claim	
Reasoning Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.	

Investigation II: Competitive inhibitors

Research question	Which sugar, lactose or sucrose, is a competitive inhibitor of the enzymatic reaction?
Experimental plan	You will collect data for three conditions: control, lactose, and sucrose.
State your hypothesis	
Justify your hypothesis	

Materials needed for this investigation:

- 2X substrate
- Dilution Buffer
- Lactose
- Sucrose
- 1X Enzyme
- Three empty 0.2 ml tubes
- P51 Molecular Fluorescence Viewer
- Micropipette and tips (2-20 µl range)
- Timer
- Fine-tipped permanent marker

Procedure:

1. Label three 0.2 ml tubes C, L, and S.
2. Add reagents to the tubes according to the table below.

Experimental condition	Control	Lactose	Sucrose
Tube label	C	L	S
2X Substrate	10 µl	10 µl	10 µl
Dilution Buffer	10 µl	-	-
Lactose	-	10 µl	-
Sucrose	-	-	10 µl

3. Place tube C in position 7 of the P51 Molecular Fluorescence Viewer, adjacent to the standard curve. Leave the P51's blue light off.
4. Read through steps 5-8 below so you are prepared for what needs to be done in the next section of this protocol.
5. Turn on the P51's blue light, and leave it on.
6. Keeping tube C in the P51 viewer, open the tube and add 5 µl of 1X enzyme directly into the liquid. Mix by slowly pipetting up and down three times.

7. As soon as the enzyme is added, each member should follow the instructions for their assigned role, as described below:

   Timekeeper

   a. Start a stopwatch.
   b. Every 15 seconds, tell the person in your group acting as the observer to collect data.
   c. Your team needs to collect data at the following time points (min:sec):

0:15	0:30	0:45	1:00	1:15	1:30	1:45	2:00

   Observer

   a. When the timekeeper tells you to collect data, estimate the brightness in the enzymatic reaction tube by comparing it to the five samples in the standard curve. Assign a brightness score (1 to 5) based on the closest match.
   b. Share the score out loud with the data recorder so they can enter it into the spreadsheet.

   Data recorder

   a. If you have someone in your group dedicated to being the data recorder, we recommend entering the data directly into a spreadsheet in a program like Google Sheets or Microsoft Excel. You can find a template here.
   b. If the same person is observing the reactions and recording the data, it might be easier to write the brightness scores down in the table provided on the next page. The times have been converted into seconds to facilitate graphical analysis.

8. After collecting data for 2 minutes, remove tube C from the P51 Molecular Fluorescence Viewer. You can discard the tube as instructed by your teacher.

9. Repeat these steps to collect data for tube L:
   a. Place tube L in position 7 of the P51, adjacent to the standard curve.
   b. Keeping tube L in the P51 viewer, open the tube and add 5 µl of 1X enzyme directly into the liquid . Mix by slowly pipetting up and down three times.
   c. Collect data every 15 seconds for 2 minutes.
   d. Remove tube L from the P51 Molecular Fluorescence Viewer.

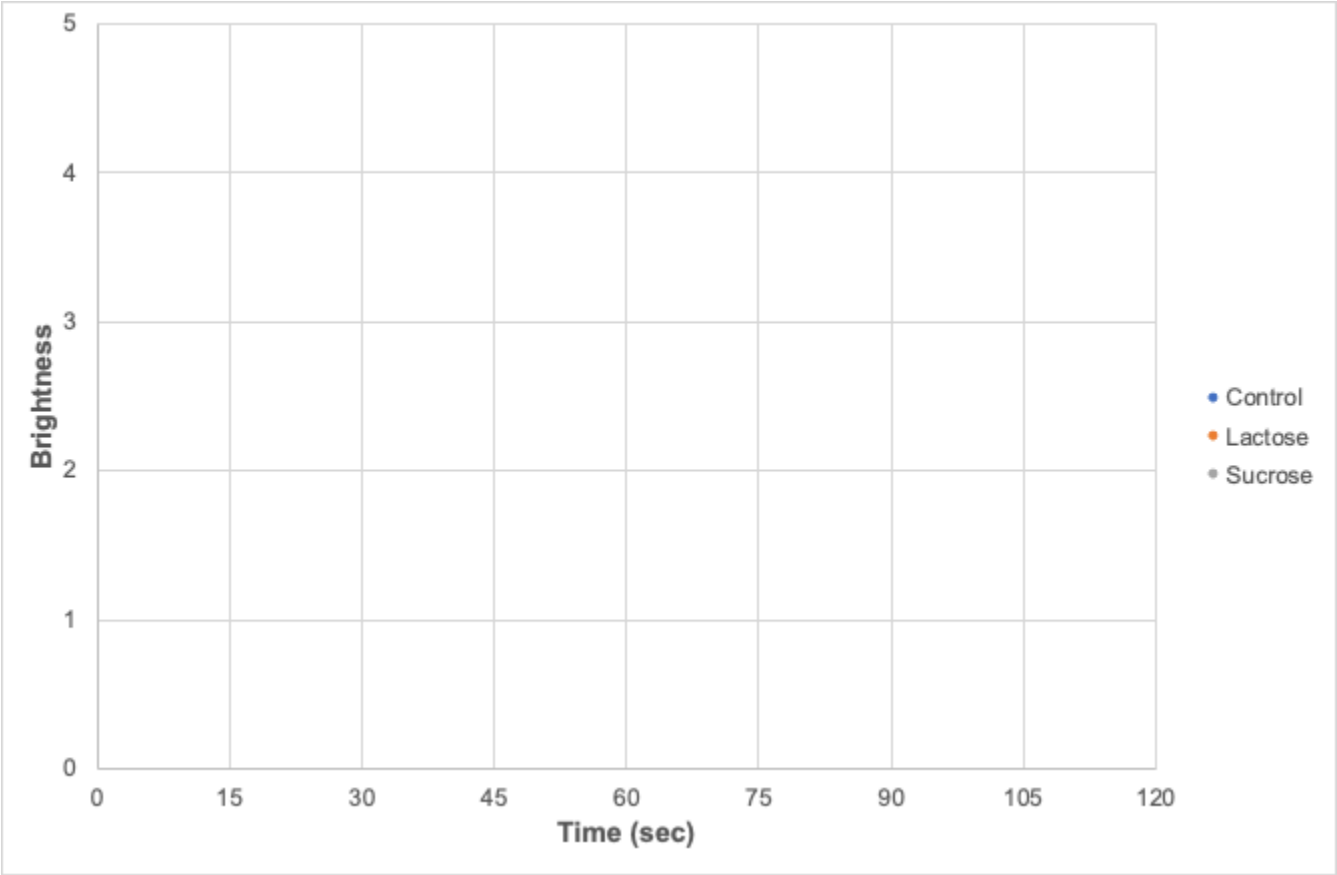
10. Repeat these steps to collect data for tube S:
    a. Place tube S in position 7 of the P51, adjacent to the standard curve.
    b. Keeping tube S in the P51 viewer, open the tube and add 5 µl of 1X enzyme directly into the liquid. Mix by slowly pipetting up and down three times.
    c. Collect data every 15 seconds for 2 minutes.
    d. Remove tube S from the P51 Molecular Fluorescence Viewer.

11. Turn off the P51's blue light.

Competitive inhibitor investigation									
Time (sec)	0	15	30	45	60	75	90	105	120
Control (Tube C)	0								
Lactose (Tube L)	0								
Sucrose (Tube S)	0								

Data analysis for Investigation II: Competitive inhibitors

1. Graph the results. The empty graph below illustrates how the graph should be set up. The X-axis should represent time, and the Y-axis should represent the brightness of the reaction as compared to your standard curve. A legend with the different experimental conditions should also be included.



2. Use the information in the graph to complete the CER table on the next page.

3. Critical thinking: What do you predict would happen to the enzymatic reaction rate if the concentration of the inhibitor was increased?

CER Table for Investigation II: Competitive inhibitors

Fill in the table based on your lab results. Refer to the CER rubric on page 27.

Question:
Which sugar acted as a competitive inhibitor of the enzymatic reaction: lactose or sucrose?

Claim Make a clear statement that answers the above question	
Evidence Provide data from the lab that supports your claim	
Reasoning Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.	

Investigation III: pH

Research question	What is the relationship between pH and the enzymatic reaction rate?
Experimental plan	You will collect data for three conditions: pH ~ 6, pH ~ 7, and pH ~ 9.
State your hypothesis	
Justify your hypothesis	

Materials needed for this investigation:

- 2X substrate
- Dilution Buffer
- NaOH
- 1X enzyme
- Three empty 0.2 ml tubes
- P51 Molecular Fluorescence Viewer
- Micropipette and tips (2-20 µl range)
- Timer
- Fine-tipped permanent marker
- Optional: pH paper

Procedure:

1. Label three 0.2 ml tubes PA, PB, and PC.
2. Prepare the substrate by adding reagents to the tubes according to the table below.

Experimental condition	pH ~6	pH ~7	pH ~9
Tube label	PA	PB	PC
2X Substrate	10 µl	10 µl	10 µl
Dilution Buffer	10 µl	5 µl	-
NaOH	-	5 µl	10 µl

3. Place tube PA in position 7 of the P51 Molecular Fluorescence Viewer, adjacent to the standard curve. Leave the P51's blue light off.
4. Read through steps 5-8 below so you are prepared for what needs to be done in the next section of this protocol.
5. Turn on the P51's blue light, and leave it on.
6. Keeping tube PA in the P51 viewer, open the tube and add 5 µl of 1X enzyme directly into the solution. Mix by slowly pipetting up and down three times.

7. As soon as the enzyme is added, each member should follow the instructions for their assigned role as described below:

   Timekeeper

   a. Start a stopwatch.
   b. Every 15 seconds, tell the person in your group acting as the observer to collect data. Your team needs to collect data at the following time points (min:sec):

0:15	0:30	0:45	1:00	1:15	1:30	1:45	2:00

   Observer

   a. When the timekeeper tells you to collect data, estimate the brightness in the enzymatic reaction tube by comparing it to the five samples in the standard curve. Assign a brightness score (1 to 5) based on the closest match.
   b. Share the score out loud with the data recorder so they can enter it into the spreadsheet.

   Data recorder

   a. If you have someone in your group dedicated to being the data recorder, we recommend entering the data directly into a spreadsheet in a program like Google Sheets or Microsoft Excel. You can find a template here.
   b. If the same person is observing the reactions and recording the data, it might be easier to write the brightness scores down in the table provided on the next page. The times have been converted into seconds to facilitate graphical analysis.

8. After collecting data for 2 minutes, remove tube PA from the P51 Molecular Fluorescence Viewer. You can discard the tube as instructed by your teacher.

9. Repeat these steps to collect data for tube PB:
   a. Place tube PB in position 7 of the P51, adjacent to the standard curve.
   b. Keeping tube PB in the P51 viewer, open the tube and add 5 µl of 1X enzyme directly into the solution. Mix by slowly pipetting up and down three times.
   c. Collect data every 15 seconds for 2 minutes.
   d. Remove tube PB from the P51 Molecular Fluorescence Viewer.

10. Repeat these steps to collect data for tube PC:
    a. Place tube PC in position 7 of the P51, adjacent to the standard curve.
    b. Keeping tube PC in the P51 viewer, open the tube and add 5 µl of 1X enzyme directly into the solution. Mix by slowly pipetting up and down three times.
    c. Collect data every 15 seconds for 2 minutes.
    d. Remove tube PC from the P51 Molecular Fluorescence Viewer.

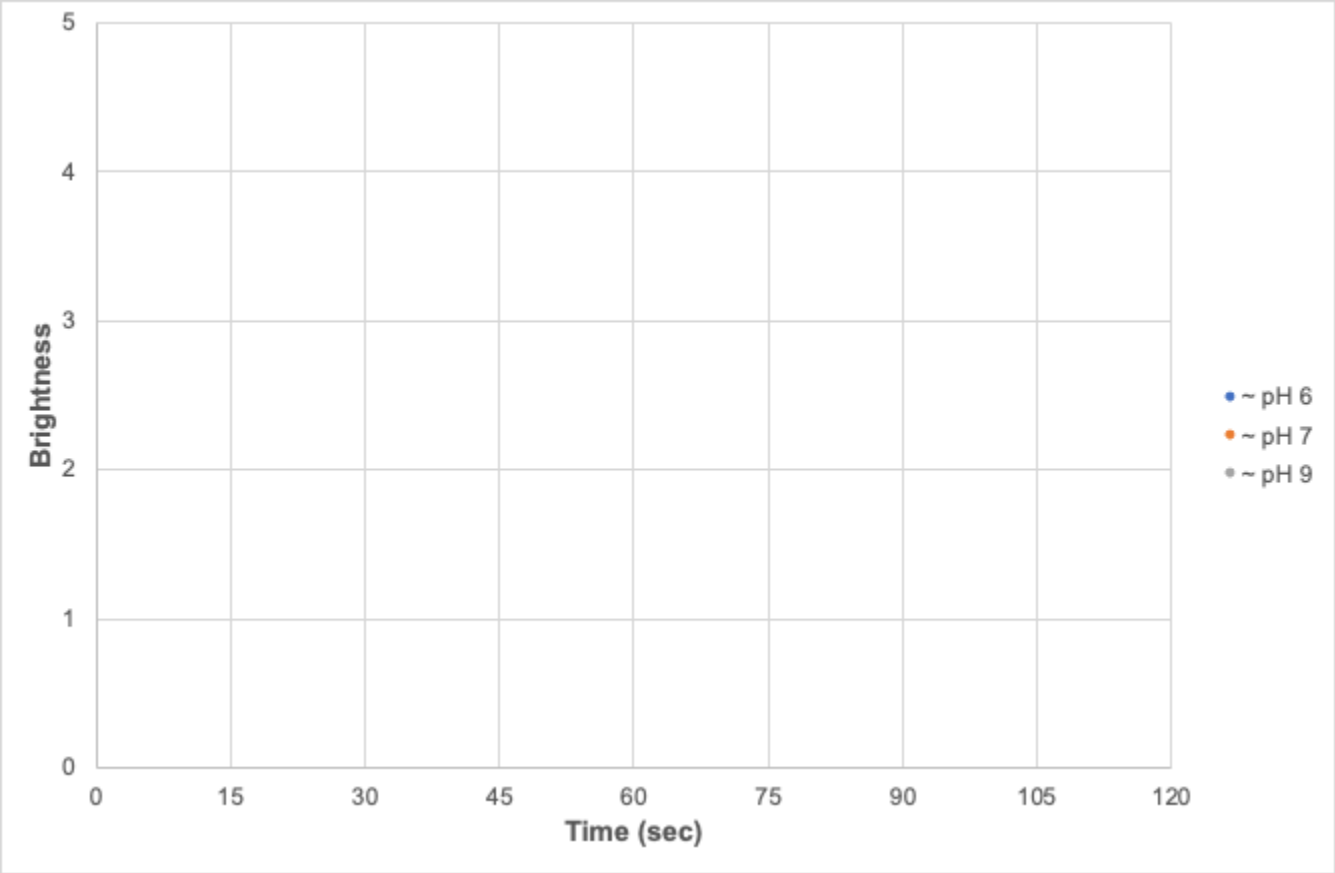
11. Turn off the P51's blue light.

12. Optional: You can measure the pH of each reaction by pipetting 2 µl of solution onto a strip of pH paper. Use the reference sheet provided with your pH paper to record the pH of each enzymatic reaction after you have collected the brightness data.

pH investigation									
Time (sec)	0	15	30	45	60	75	90	105	120
pH ~ 6 (Tube PA)	0								
pH ~ 7 (Tube PB)	0								
pH ~ 9 (Tube PC)	0								

Data analysis for Investigation III: pH

1. Graph the results. The empty graph below illustrates how the graph should be set up. The X-axis should represent time, and the Y-axis should represent the brightness of the reaction as compared to your standard curve. A legend with the different experimental conditions should also be included.



2. Use the information in the graph to complete the CER table on the next page.
3. Critical thinking: In this lab, you only increased the pH. Predict what would happen if you lowered the pH of the reaction by adding acid. Explain your reasoning.

CER Table for Investigation III: pH

Fill in the table based on your lab results. Refer to the CER rubric on page 27.

Question:
What is the relationship between pH and the enzymatic reaction rate?

Claim Make a clear statement that answers the above question	
Evidence Provide data from the lab that supports your claim	
Reasoning Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.	

Investigation IV: Temperature

Research question	What is the relationship between temperature and the enzymatic reaction rate?
Experimental plan	You will collect data for four temperature conditions: ice bath, room temperature, 55 °C, and 75 °C or higher.
State your hypothesis	
Justify your hypothesis	

Materials needed for this investigation:

- 2X substrate
- Dilution Buffer
- 1X Enzyme
- Eight empty 0.2 ml tubes
- P51 Molecular Fluorescence Viewer
- Micropipette and tips (2-20 µl range)
- Timer
- Fine-tipped permanent marker
- Ice bath
- Heat source
- Optional: thermometer

Procedure:

1. Prepare an ice bath by adding a small amount of water to crushed ice.
2. If using a digital heat block or a water bath as a heat source, set it to 55 °C now. If using a thermal cycler in heat block mode, you do not need to prepare the heat source in advance as thermal cyclers heat up very quickly.
3. Label four 0.2 ml tubes TA, TB, TC, and TD.
4. Prepare the substrate by adding reagents to the tubes according to the table below:

Experimental condition	Ice bath	Room temp.	55 °C	75 °C or higher
Tube label	TA	TB	TC	TD
2X Substrate	10 µl	10 µl	10 µl	10 µl
Dilution Buffer	10 µl	10 µl	10 µl	10 µl

5. Label four 0.2 ml tubes ETA, ETB, ETC, ETD.
6. Add enzyme to the tubes according to the table below:

Experimental condition	Ice bath	Room temp.	55 °C	75 °C or higher
Tube label	ETA	ETB	ETC	ETD
1X enzyme	8 µl	8 µl	8 µl	8 µl

7. Incubate tubes TA and ETA in the ice bath for two minutes. If you have access to a thermometer, record the temperature of the ice bath.
8. Place tube TA in position 7 of the P51 Molecular Fluorescence Viewer, adjacent to the standard curve. Leave off the P51's blue light.
9. Read through steps 10-13 below so you are prepared for what needs to be done in the next section of this protocol.
10. Turn on the P51's blue light, and leave it on.
11. Without removing tube TA from the P51, quickly add 5 µl of enzyme from tube ETA directly into the solution in tube TA. Mix by slowly pipetting up and down three times.
12. As soon as the enzyme is added, each member should follow the instructions below for their assigned role:

Timekeeper

a. Start a stopwatch.
b. Every 15 seconds, tell the person in your group acting as the observer to collect data. Your team needs to collect data at the following time points (min:sec):

0:15	0:30	0:45	1:00	1:15	1:30	1:45	2:00

Observer

a. When the timekeeper tells you to collect data, estimate the brightness of the enzymatic reaction tube by comparing it to the five samples in the standard curve. Assign a brightness score (1 to 5) based on the closest match.
b. Share the score out loud with the data recorder so they can enter it into the spreadsheet.

Data recorder

a. If you have someone in your group dedicated to being the data recorder, we recommend entering the data directly into a spreadsheet in a program like Google Sheets or Microsoft Excel. You can find a template here.
b. If the same person is observing the reactions and recording the data, it might be easier to write the brightness scores down in the table provided on the next page. The times have been converted into seconds to facilitate graphical analysis.

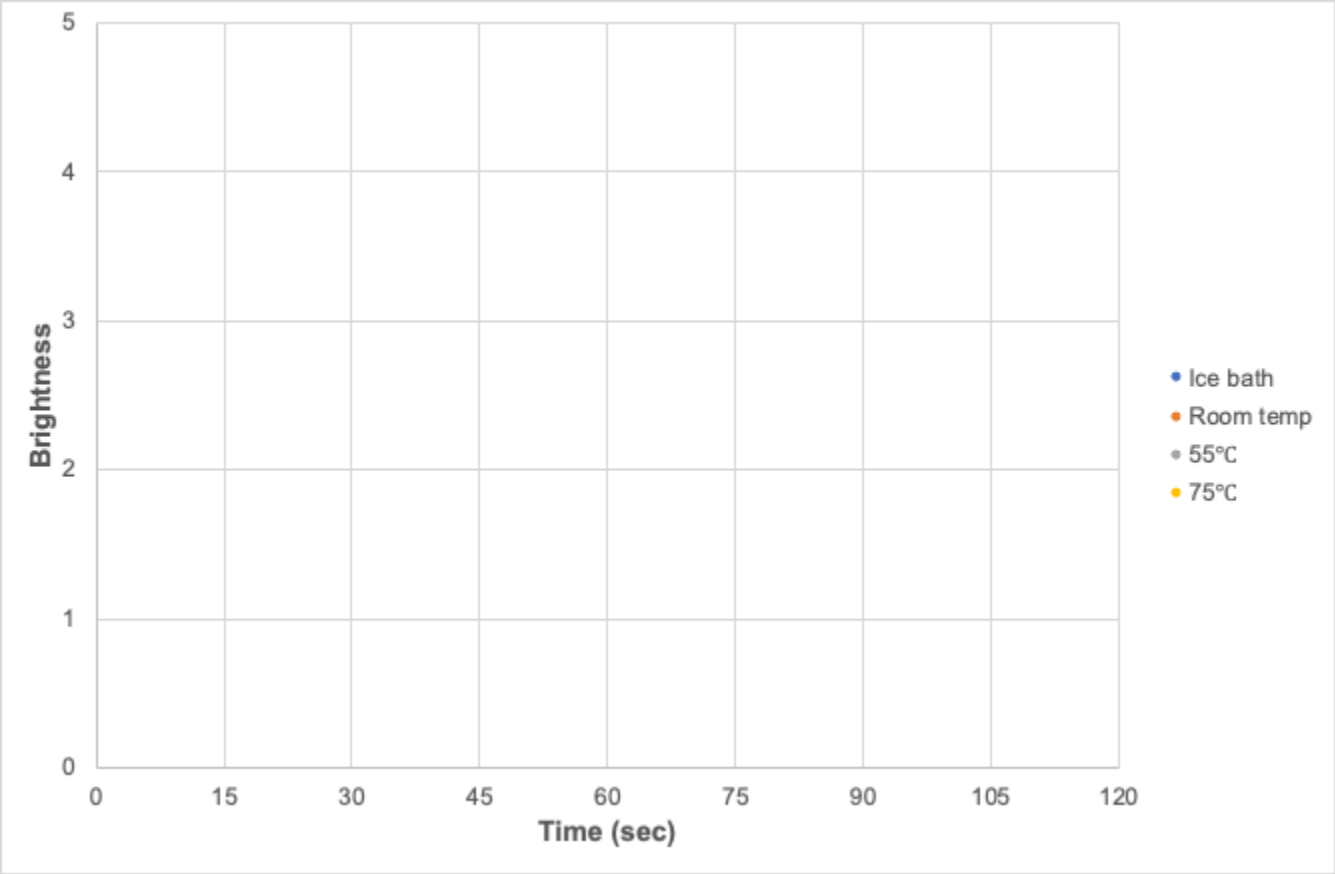
13. After collecting data for 2 minutes, remove tube TA from the P51 Molecular Fluorescence Viewer. You can discard the tube as instructed by your teacher.

14. Repeat these steps to collect data for the room temperature condition:
    a. Incubate tubes TB and ETB at room temperature for two minutes. If you have access to a thermometer, record the ambient temperature.
    b. Place tube TB in position 7 of the P51, adjacent to the standard curve.
    c. Without removing tube TB from the P51, quickly add 5 µl of enzyme from tube ETB directly into the solution in tube TB. Mix by slowly pipetting up and down three times
    d. Collect data every 15 seconds for 2 minutes.
    e. Remove tube TB from the P51 Molecular Fluorescence Viewer.
15. Repeat these steps to collect data for the 55 °C condition:
    a. Check to make sure your heat source is at 55 °C, and record the temperature.
    b. Incubate tubes TC and ETC at 55 °C for two minutes.
    c. Place tube TC in position 7 of the P51, adjacent to the standard curve.
    d. Without removing tube TC from the P51, quickly add 5 µl of enzyme from tube ETC directly into the solution in tube TC. Mix by slowly pipetting up and down three times
    e. Collect data every 15 seconds for 2 minutes.
    f. Remove tube TC from the P51 Molecular Fluorescence Viewer.
16. Repeat these steps to collect data for the 75 °C or higher condition:
    a. Check to make sure your heat source is at 75 °C or higher, and record the temperature.
    b. Incubate tubes TD and ETD at 75 °C or higher for two minutes.
    c. Place tube TD in position 7 of the P51, adjacent to the standard curve.
    d. Without removing tube TD from the P51, quickly add 5 µl of enzyme from tube ETD directly into the solution in tube TD. Mix by slowly pipetting up and down three times.
    e. Collect data every 15 seconds for 2 minutes.
    f. Remove tube TD from the P51 Molecular Fluorescence Viewer.
17. Turn off the P51's blue light.

Temperature investigation									
Time (sec)	0	15	30	45	60	75	90	105	120
Ice bath (Tube TA)	0								
Room temp. (Tube TB)	0								
55 °C (Tube TC)	0								
75 °C or higher (Tube TD)	0								

Data analysis for Investigation IV: Temperature

1. Graph the results. The empty graph below illustrates how the graph should be set up. The X-axis should represent time, and the Y-axis should represent the brightness of the reaction as compared to your standard curve. A legend with the different experimental conditions should also be included.



2. Use the information in the graph to complete the CER table on the next page.
3. Critical thinking: If you were able to isolate the enzyme from your 75 °C trial and test it again, it is likely that you would observe little enzymatic activity. Explain why this would be the case.

CER Table for Investigation IV: Temperature

Fill in the table based on your results from the lab. Refer to the CER rubric on page 27.

Question:
What is the relationship between temperature and the enzymatic reaction rate?

Claim Make a clear statement that answers the above question	
Evidence Provide data from the lab that supports your claim	
Reasoning Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.	

Instructor guide

*For additional information on alignment to state standards please contact support@minipcr.com

Expected results

Investigation I: Substrate and enzyme concentration

- Higher enzyme concentration will cause clear increases in reaction rate.
- Changing substrate concentration will change the rate of the reaction, but also the total amount of product. This means that the final brightness of the tube will change as well.

Investigation II: Competitive inhibitors

- Lactose contains a terminal galactose, so it acts as a competitive inhibitor by occupying the active site of the enzyme and preventing the galactose substrate from binding. This will lead to a decrease in the reaction rate.
- Sucrose does not contain a terminal galactose and should have no effect on the reaction rate.

Investigation III: pH

- The reaction rate is greatest between pH 6-7.
- Above pH 9 the reaction is completely inhibited.

Investigation IV: Temperature

- Of the recommended temperatures to test, 55 °C should show the fastest reaction rate.
- Lower temperatures should show a slower reaction rate.
- Above 75 °C the enzyme denatures and the reaction is completely inhibited.

For technical support, contact support@minipcr.com

For answers to the student questions, email answers@minipcr.com
Please include in the body of the email:

- The name of the lab
- Your name, school, and job title

Differentiation

This activity is well suited to a student-driven inquiry approach. Instead of providing students with the experimental parameters to test for each investigation, you can modify this activity and allow students to determine the conditions they test. You can also invite students to propose other environmental conditions that they wish to investigate.

Learning goals and skills developed

Student learning goals

- Relate the role of substrates and enzymes in reactions
- Demonstrate how changing conditions affect enzyme reaction rates.

Scientific inquiry skills

- Identify or pose a testable question
- Formulate hypotheses
- Identify dependent and independent variables and appropriate experimental controls
- Follow detailed experimental protocols
- Create tables or graphs to present their results
- Interpret data presented in a chart or table
- Use data to evaluate a hypothesis
- Make a claim based in scientific evidence
- Use reasoning to justify a scientific claim

Molecular biology skills

- Micropipetting
- Fluorescence detection

Standards alignment

Next Generation Science Standards (NGSS)

SCIENCE & ENGINEERING PRACTICES	DISCIPLINARY CORE IDEAS	CROSSCUTTING CONCEPTS
• Asking Questions and Defining Problems • Planning and Carrying Out Investigations • Analyzing and Interpreting Data • Constructing Explanations and Designing Solutions • Engaging in Argument from Evidence • Obtaining, Evaluating, and Communicating Information	• LS1.A: From Molecules to Organisms: Structures and Processes	• Patterns • Cause and Effect • Structure and Function • Stability and Change

AP Biology

COURSE CONTENT	
Unit 1: Chemistry of Life	
Topic 1.4: Properties of Biological Macromolecules • Enduring Understanding SYI-1: Living systems are organized in a hierarchy of structural levels that interact.	
Learning Objective SYI-1.B: Describe the properties of the monomers and the type of bonds that connect the monomers in biological macromolecules.	
SYI-1.B.2	Structure and function of polymers are derived from the way their monomers are assembled. b. In proteins, the specific order of amino acids in a polypeptide (primary structure) determines the overall shape of the protein. Amino acids have directionality, with an amino (NH2) terminus and a carboxyl (COOH) terminus. The R group of an amino acid can be categorized by chemical properties (hydrophobic, hydrophilic, or ionic), and the interactions of these R groups determine structure and function of that region of the protein.
Topic 1.5: Structure and Function of Biological Macromolecules • Enduring Understanding SYI-1: Living systems are organized in a hierarchy of structural levels that interact.	
Learning Objective SYI-1.C: Explain how a change in the subunits of a polymer may lead to changes in structure or function of the macromolecule.	
SYI-1.C.1	Directionality of the subcomponents influences structure and function of the polymer. d. Proteins have primary structure determined by the sequence order of their constituent amino acids, secondary structure that arises through local folding of the amino acid chain into elements such as alpha-helices and beta-sheets, tertiary structure that is the overall three-dimensional shape of the protein and often minimizes free energy, and quaternary structure that arises from interactions between multiple polypeptide units. The four elements of protein structure determine the function of a protein.
Topic 1.6: Nucleic Acids • Enduring Understanding IST-1: Heritable information provides for continuity of life.	
Learning Objective IST-1.A: Describe the structural similarities and differences between DNA and RNA.	
IST-1.A.1	DNA and RNA molecules have structural similarities and differences related to their function. a. Both DNA and RNA have three components—sugar, a phosphate group, and a nitrogenous base—that form nucleotide units that are connected by covalent bonds to form a linear molecule with 5' and 3' ends, with the nitrogenous bases perpendicular to the sugar-phosphate backbone. b. The basic structural differences between DNA and RNA include the following: i. DNA contains deoxyribose and RNA contains ribose. ii. RNA contains uracil and DNA contains thymine. iii. DNA is usually double stranded; RNA is usually single stranded. iv. The two DNA strands in double-stranded DNA are antiparallel in directionality
Unit 3: Cellular Energetics	
Topic 3.1: Enzyme Structure • Enduring Understanding ENE-1: The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	
Learning Objective ENE-1.D: Describe the properties of enzymes.	
ENE-1.D.1	The structure of enzymes includes the active site that specifically interacts with substrate molecules.
ENE-1.D.2	For an enzyme-mediated chemical reaction to occur, the shape and charge of the substrate must be compatible with the active site of the enzyme.

Topic 3.2: Enzyme Catalysis • Enduring Understanding ENE-1: The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	
Learning Objective ENE-1.E: Explain how enzymes affect the rate of biological reactions.	
ENE-1.E.1	The structure and function of enzymes contribute to the regulation of biological processes. a. Enzymes are biological catalysts that facilitate chemical reactions in cells by lowering the activation energy.
Topic 3.3: Environmental Impacts on Enzyme Function • Enduring Understanding ENE-1: The highly complex organization of living systems requires constant input of energy and the exchange of macromolecules.	
Learning Objective ENE-1.F: Explain how changes to the structure of an enzyme may affect its function.	
ENE-1.F.1	Change to the molecular structure of a component in an enzymatic system may result in a change of the function or efficiency of the system. a. Denaturation of an enzyme occurs when the protein structure is disrupted, eliminating the ability to catalyze reactions. b. Environmental temperatures and pH outside the optimal range for a given enzyme will cause changes to its structure, altering the efficiency with which it catalyzes reactions.
ENE-1.F.2	In some cases, enzyme denaturation is reversible, allowing the enzyme to regain activity.
Learning Objective ENE-1.G: Explain how the cellular environment affects enzyme activity.	
ENE-1.G.1	Environmental pH can alter the efficiency of enzyme activity, including through disruption of hydrogen bonds that provide enzyme structure.
ENE-1.G.2	The relative concentrations of substrates and products determine how efficiently an enzymatic reaction proceeds.
ENE-1.G.3	Higher environmental temperatures increase the speed of movement of molecules in a solution, increasing the frequency of collisions between enzymes and substrates and therefore increasing the rate of reaction.
ENE-1.G.4	Competitive inhibitor molecules can bind reversibly or irreversibly to the active site of the enzyme. Noncompetitive inhibitors can bind allosteric sites, changing the activity of the enzyme.
SCIENCE PRACTICES	
1. Concept Explanation: Explain biological concepts, processes, and models presented in written format.	
A	Describe biological concepts and/or processes.
B	Explain biological concepts and/or processes.
C	Explain biological concepts, processes, and/or models in applied contexts.
3. Questions and Methods: Determine scientific questions and methods.	
A	Identify or pose a testable question based on an observation, data, or a model.
B	State the null and alternative hypotheses, or predict the results of an experiment.
C	Identify experimental procedures that are aligned to the question, including a) Identifying dependent and independent variables. b) Identifying appropriate controls. c) Justifying appropriate controls
D	Make observations, or collect data from representations of laboratory setups or results. (Lab only; not assessed)
E	Propose a new/next investigation based on a) An evaluation of the evidence from an experiment. b) An evaluation of the design/methods.

4. Representing and Describing Data: Represent and describe data.	
A	Construct a graph, plot, or chart (X,Y; Log Y; Bar; Histogram; Line, Dual Y; Box and Whisker; Pie). a) Orientation b) Labeling) Units. d) Scaling e) Plotting f) Type g) Trend line
B	Describe data from a table or graph, including a) Identifying specific data points. b) Describing trends and/or patterns in the data. c) Describing relationships between variables.
6. Argumentation: Develop and justify scientific arguments using evidence.	
A	Make a scientific claim.
B	Support a claim with evidence from biological principles, concepts, processes, and/or data.
C	Provide reasoning to justify a claim by connecting evidence to biological theories.
D	Explain the relationship between experimental results and larger biological concepts, processes, or theories.
E	Predict the causes or effects of a change in, or disruption to, one or more components in a biological system based on a) Biological concepts or processes. b) A visual representation of a biological concept, process, or model. c) Data.

Biology Texas Essential Knowledge and Skills (TEKS)

HIGH SCHOOL BIOLOGY SCIENCE CONCEPTS	
7. Gene expression	
D	Discuss the importance of molecular technologies such as polymerase chain reaction (PCR), gel electrophoresis, and genetic engineering that are applicable in current research and engineering practices
11. Metabolic processes and enzymes	
B	Investigate and explain the role of enzymes in facilitating cellular processes
HIGH SCHOOL SCIENCE AND ENGINEERING PRACTICES	
SEP 1. Investigations	
A	Ask questions and define problems based on observations or information from text, phenomena, models, or investigations
B	Apply scientific practices to plan and conduct descriptive, comparative, and experimental investigations and use engineering practices to design solutions to problems
C	Use appropriate safety equipment and practices during laboratory, classroom, and field investigations as outlined in Texas Education Agency-approved safety standards
D	Use appropriate tools such as microscopes, slides, Petri dishes, laboratory glassware, metric rulers, digital balances, pipettes, filter paper, micropipettes, gel electrophoresis and polymerase chain reaction (PCR) apparatuses, microcentrifuges, water baths, incubators, thermometers, hot plates, data collection probes, test tube holders, lab notebooks or journals, hand lenses, and models, diagrams, or samples of biological specimens or structures
E	Collect quantitative data using the International System of Units (SI) and qualitative data as evidence
F	Organize quantitative and qualitative data using scatter plots, line graphs, bar graphs, charts, data tables, digital tools, diagrams, scientific drawings, and student-prepared models
SEP 2. Data	
B	Analyze data by identifying significant statistical features, patterns, sources of error, and limitations
SEP 3. Explanations	
A	Develop explanations and propose solutions supported by data and models and consistent with scientific ideas, principles, and theories
B	Communicate explanations and solutions individually and collaboratively in a variety of settings and formats
C	Engage respectfully in scientific argumentation using applied scientific explanations and empirical evidence
SEP 4. Scientist contributions	
A	Analyze, evaluate, and critique scientific explanations and solutions by using empirical evidence, logical reasoning, and experimental and observational testing, so as to encourage critical thinking by the student

Biotechnology Texas Essential Knowledge and Skills (TEKS)

HIGH SCHOOL BIOTECHNOLOGY CONCEPTS	
3. Scientific methods	
E	Plan and implement investigative procedures, including asking questions, formulating testable hypotheses, and selecting, handling, and maintaining appropriate equipment and technology
F	Collect data individually or collaboratively, make measurements with precision and accuracy, record values using appropriate units, and calculate statistically relevant quantities to describe data, including mean, median, and range
G	Demonstrate the use of course apparatus, equipment, techniques, and procedures
H	Organize, analyze, evaluate, build models, make inferences, and predict trends from data
7. Genetics	
G	Describe the structure and function of proteins, including 3D folding, enzymes, and antibodies
10. Procedure performance	
B	Practice measuring volumes and weights to industry standards
D	Demonstrate proficiency pipetting techniques
F	Document laboratory results
11. Prepare solutions	
C	Calculate and prepare a dilution series

Biotechnician Assistant Credentialing Exam (BACE)

KNOWLEDGE PORTION OF EXAM
Biochemistry/chemistry
• Demonstrate knowledge of enzymes and reaction rates • Describe protein structure and function
PRACTICAL PORTION OF EXAM
Biotechnology skills
• Accurately measure liquids using micropipettes and serological pipets • Properly prepare solutions, buffers, and media • Properly perform a serial dilution
Laboratory equipment: demonstrate proper and safe use of equipment
• Micropipettes & serological pipets
Research and scientific method
• Analyze and interpret data, including the use of statistical analysis

Common Core ELA/Literacy Standards (9-10)

RST.9-10.1	Cite specific textual evidence to support analysis of science and technical text, attending to the precise details of explanations or descriptions.
RST.9-10.3	Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks, attending to special cases or exceptions defined in the text.
RST.9-10.4	Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 9-10 texts and topics.
RST.9-10.5	Analyze the structure of the relationships among concepts in a text, including relationships among key terms (*E.g*. force, friction, reaction force, energy).
RST9-10.9	Compare and contrast findings presented in a text to those from other sources (including their own experiments), noting when the findings support or contradict previous explanations or accounts.
WHST.9-10.1	Write arguments focused on discipline-specific content.
WHST.9-10.2	Write informative/explanatory texts, including the narration of historical events, scientific procedures/experiments, or technical processes.
WHST.9-10.9	Draw evidence from informational text to support analysis, reflection, and research.