I’ve Got That Sinking Feeling

An Investigation of Enzymes

Teacher Materials

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**Please consider adapting this lab to include some student-centered investigation. Some suggestions, ideas, and tips can be found in a separate document called "Student-Centered Investigation”.*

Last updated: 9/21/18

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Learning Goals, Objectives, and Skills

Students Learning Goals:

- Students will understand the role of enzymes in chemical reactions.
- Students will understand the basic steps of enzyme catalyzed reactions.
- Students will understand some of the factors that can influence the rate of enzyme-catalyzed reactions.

Student Learning Objectives:

- Students will articulate the function of catalase in breaking down hydrogen peroxide and identify the reactants and products of this chemical reaction.
- Students will explain the role of enzymes as catalysts that lower the activation energy of biochemical reactions.
- Students will measure the effect of enzyme concentration and other factors on the rate of catalase mediated chemical reactions.

Scientific Inquiry Skills:

- Students will pose questions and form hypotheses.
- Students will design and conduct scientific investigations.
- Students will make measurements and record data.
- Students will use mathematical operations to analyze and interpret data.
- Students will generate tables and graphs to present their data.
- Students will use experimental data to make conclusions about the initial question and to support or refute the stated hypothesis.
- Students will follow laboratory safety rules and regulations.

Laboratory Technical Skills:

- Students will demonstrate proper use of micropipettes.

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Instructor Planning Guide

Experimental Timing:

From start to finish this lab can be completed in a single 45-50 minute class period. It is recommended that the pre-lab discussion and teacher demonstration of the procedure be completed the day before the lab.

Specialized Equipment:

- p20 micropipettes*
- p200 micropipettes*
- p1000 micropipettes*
- 24-well plates*

* See Instructor Preparation Guide for substitutions

Ordering Information:

This lab was developed using Fisher Scientific products and reagents. Catalog numbers in the Teacher Advanced Preparation Materials list when possible. Hydrogen peroxide can be purchased from a drug store or grocery store. ⓘ**Tip: Hydrogen peroxide can often be found at dollar stores.**

Procedure Tips:

1. Before starting the I’ve Got That Sinking Feeling experiment, ask students to check their materials list to make sure they have everything.

2. Demonstrate how to set up the 24-well plates, the procedure for applying catalase to the disks, and how the disk will sink and float during the assay. In order to get the disk off of the forceps when inserting it into the vial, students should deposit the disk just under the surface of the hydrogen peroxide solution.

3. Remind students to make all dilutions using the 100% catalase stock solution.

4. To avoid a buildup of catalase, students should use fresh hydrogen peroxide after performing 3 trials at each experimental condition (concentration).

5. To prevent contamination, students must wipe the forceps clean between each trial to remove any catalase.

6. During the assay it is fine if the disk does not sink all the way to bottom of the vial before it begins to rise to the top of the solution. Students should just time from the moment they release the disk into the vial to the moment it rises to the top.

7. At the 0% catalase condition, the disk will not rise to the surface of the solution during the course of the experiment. However, since hydrogen peroxide breaks down when exposed to light, students may see the disk rise over an extended period of time (overnight).

Teaching Tips:

While students are working on their lab generate a table on the board or projector to collect class data.

Record the average time (sec) each group obtained for each experimental condition and calculate a class average. You could also have students perform a t-test and calculate a p-value.

	Average Time (sec) for Disk to Sink and Rise							
	Group Number							
Catalase Concentration	1	2	3	4	5	....	15	Class Average
100%								
80%								
60%								
40%								
20%								

Safety Considerations:

- Gloves, lab coats and eye protection should be used whenever possible, as a part of good laboratory practice. I deleted the one about lights
- Always wash hands thoroughly after handling biological materials or reagents.
- Obtain the Material Safety Data Sheets (MSDS) available from the suppliers, and follow all safety precautions and disposal directions as described in the MSDS.
- Check with your school’s lab safety coordinator about proper disposal of all reagents.

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Instructor Preparation Guide

Materials: This guide assumes 30 students, working in groups of two, for a totally of 15 groups.

Materials for Advanced Preparation:	
300 mL	hydrogen peroxide solution (3.0%)°
100 mg	catalase (Fisher Catalog # S75082)
11 L	distilled water
1	250 mL flask or bottle
1	4 L flask or bottle
1	1 L graduated cylinder
1	100 mL graduated cylinder
15	15 mL conical tubes with lids
15	500 mL flasks or bottles
15	200 mL opaque bottles (Fisher Catalog # 03-313-412)°
1	permanent marker

▲**Caution: Hydrogen peroxide is light-sensitive, which is why it's important to store the solution in opaque containers**

Student Workstation:		Common Workstation:
150 mL	hydrogen peroxide solution (0.3%)	Extra hydrogen peroxide (0.3%)
5 mL	catalase stock solution (0.4 mg/mL)	Extra dH_2O
5mL	distilled water	
1	p20 micropipette with tips	
1	p200 micropipette with tips	
1	p1000 micropipette with tips	
1	24-well plate (Fisher Catalog # 07-201-590)	
2	20 mL clear vials (Fisher Catalog # 03-337-14)	
1	single hole paper punch	
1	forceps	
1 sheet	filter paper (Fisher Catalog # 09-825B)	
1	stop watch or timer	
1	permanent marker	
2+	paper towels	

Easy Substitutions:

- Potatoes, liver or yeast can be used as a source of catalase.
- Graduated 1-mL transfer pipettes can be substituted for the p1000 and p200 micropipettes and tips.
- Microcentrifuge tubes or small containers (such as bottle caps) can be substituted for the 24-well plate. Each group will need 6 containers.
- Recycle fruit fly vials and use them as the reaction vials.
- If a p20 micropipette is not available, dip the disk into the solution and then touch it to a paper towel to remove the excess liquid.
  ⓘ**Tip: Don't forget to modify the student hand out to reflect any substitutions.**

Set-up Calendar:

2 weeks before lab:

- Check supplies and order any needed materials.
- If making any substitutions to the supply list, edit the student protocol accordingly.

1 day before lab

- Set up student lab stations with all durable materials according to the materials listed above.

Morning of lab:

- Prepare 3 L of a 0.3% hydrogen peroxide
  1. Mix 300 mL of 3% hydrogen peroxide with 2700 mL of water in a 4-L flask.
  2. Aliquot 150 mL of the 0.3% solution into 250-mL opaque bottles. Prepare 1 bottle per lab group. ⓘ**Tip: Aliquot a few extra in the event of spills.**
- Put one bottle at each station.
- Prepare 250 mL of 100% catalase stock solution. The 100% solution is actually an artificial designation. It gives the students a starting point for making dilutions. When preparing this solution teachers should start with about 0.004 g of catalase in 250 ml of water and then test it as described below. If you do not have a balance that can measure 0.004g it is a very small "pinch" of powder. Unfortunately, making this solution is trial and error but the key is to have the 100% catalase strong enough to get a measurable rate when the disk is dropped into the hydrogen peroxide.
- Test the solutions:
  1. Place 10 μL of the catalase (100%) stock solution on a filter paper disk and dropping it into a vial of 20 mL of 0.3% hydrogen peroxide solution. The disk should sink and then rise to the surface of the solution in 10 seconds or less.

2. If the disk does not sink, dilute the catalase solution (1:4) and test again. Alternatively you can dilute the hydrogen peroxide solution incrementally by 50% until you reach the desire reaction rate.

▲Caution: It is critical to test the solutions prior to the lab, because students will obtain much better results if the stock solutions aren't too strong.

- Aliquot 5 mL of the 100% catalase stock solution into conical tubes. Prepare one tube per lab group. **ⓘTip: Aliquot a few extra in the event of spills.**
- Put one tube at each station.

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Answers to Student Questions

Protocol-Embedded:

p. 3: The tube with the catalase solution would contain bubbles, the other tube would not.

Completed dilution table:

Final quantity needed (μL)	Final catalase solution concentration	Microliters (μL) of 0.4 mg/mL catalase stock solution	Microliters (μL) of deionized water
500	100%	500	0
500	80%	400	100
500	60%	300	200
500	40%	200	300
500	20%	100	400
500	0%	0	500

Step 4 (p. 5): About half full

Step 17 (p. 6): The disk should not have moved

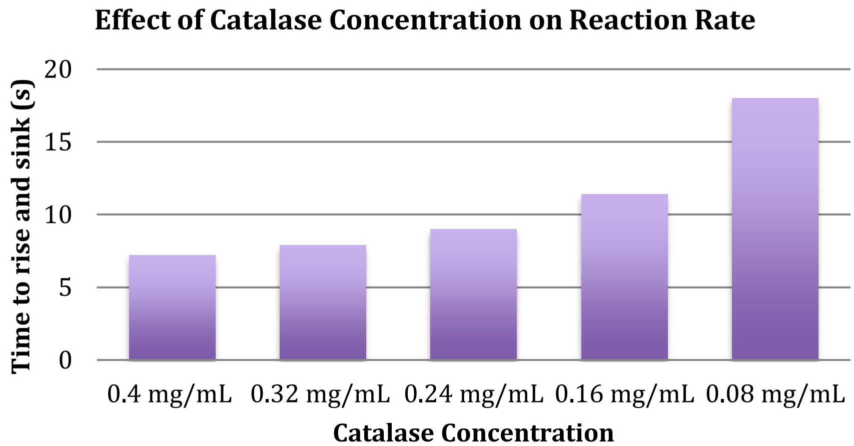
Pre-Lab:

1. Enzymes catalyze (lower the potential energy of) chemical reactions that occur within living things and are necessary for life processes.
2. Enzymes tend to be substrate-specific—they only work on specific substrates.
3. Because enzymes are not consumed by the reactions they catalyze, so they can be used again and again.
4. Catalase catalyzes the reaction to break down hydrogen peroxide.
5. Dependent: time that it takes the disk to sink and rise; Independent: catalase concentration; Control: distilled water set-up
6. *Sample answer:* The more concentrated the catalase, the faster the reaction rate (the less time it will take for the disk to sink and rise).

Post-Lab and Analysis

1. Because the oxygen produced during the reaction makes the disk more rise
2. Sample data table and graph:

	Time (s) for Disk to Sink and Rise to Surface			
Catalase concentration	**Trial 1**	**Trial 2**	**Trial 3**	**Average Time**
100%	6.6	7.7	7.3	7.2
80%	7.4	7.9	8.4	7.9
60%	8.7	9.3	8.9	9.0
40%	12.3	10.7	11.2	11.4
20%	19.9	16.6	17.5	18.0



3. The more concentrated the enzyme, the faster the reaction rate. This agrees with my hypothesis.
4. Because more enzyme was available to facilitate the hydrogen peroxide reaction.
5. I'd predict the graph on the right, in which the reaction rate levels off. I'd make this prediction because at some point, the amount of enzyme will surpass the available substrate, making additional enzyme have no further effect on the rate. In other words, some other factor will become limiting.
6. 1. There could have been human timing errors (e.g., failing to start and stop the timer with accuracy)

   2. There could have been some kind of effect from "old" hydrogen peroxide. If it contained catalase from previous trials that might speed up the reaction

   3. If using the "dip" method for transferring solution to the disks, there could have been variation in the amount of solution transferred. Some disks might have more solution on them than others
7. Yes, because the reaction releases oxygen, which makes the disks float. The faster the reaction, the more bubbles are released, and the faster the disk will rise to the surface.
8. If catalase were kept constant, then there would be an increase in the rate with increased concentration of hydrogen peroxide, assuming that enough catalase was present. The amount of catalase could be a limiting factor if the ratio of the number of molecules of hydrogen peroxide to molecules of catalase is high.

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Post-Lab Extension Activities

Student Oral Presentation:

Students can report the findings of their student-centered investigations to the class using a PowerPoint presentation that includes the following information:

- Experimental question—what you hope to learn from performing the experiment.
- Hypothesis—a testable, proposed answer to the experimental question based on prior knowledge.
- Experimental system and data collection methods—flowchart of how the experiment was performed and how data was collected. This should NOT include a detailed summary.
- Results—observations, data tables, figures, etc.
- Conclusions—should the hypothesis be accepted or rejected as supported by key data.

Online resource for effective PowerPoint presentations:

http://office.microsoft.com/en-us/powerpoint-help/tips-for-creating-and-delivering-an-effective-presentation-HA010207864.aspx

Student Lab Report:

Students can report the findings of their student-centered investigations through a written lab report. Your school may have its own lab report format, but generally lab reports include the following information:

- Title—brief summary reflecting the factual content of the investigation.
- Introduction—includes questions being answered, hypothesis and background information.
- Materials—list of supplies needed to perform the lab.
- Procedure—step-by-step procedure (with enough detail so someone could repeat the experiment).
- Results—observations, data tables, figures, etc. and a brief narrative summary of results.
- Conclusion—explanation supported by evidence for whether the hypothesis should be accepted or rejected.

Online resources for writing lab reports:

http://www.mhhe.com/biosci/genbio/maderinquiry/writing.html

http://www.ncsu.edu/labwrite/

Student Writing Exercise:

Ask students to read the online article *Enzymes Will Play a Key Role in Development of Sustainable Society, Expert Says* (http://www.sciencedaily.com/releases/2011/05/110502092249.htm) and write a brief paragraph addressing the following questions:

- List several different enzymes and their function?
- What are some processed foods or commercial processes that rely on enzymatic reactions?

- How are enzymes used in medicine?
- How is Professor Van Berkel hoping to use enzymes for a more sustainable society?
- How can industry increase the production of rare enzymes?

Enzymes will play a key role in development of sustainable society, expert says

June 20, 2011 — Enzymes play a central role in the chemistry of living nature. They facilitate our digestion, for example, and often determine the difference between sick or healthy organisms. Professor Willem van Berkel, Professor of Molecular Enzymology at Wageningen University, goes a step further. He expects enzymes to play a key role in the development of a sustainable society.

Based on genes*, it can be predicted that there are around 25,000 enzymes. Of these enzymes, only 5,000 have been characterized, so there are a great many that we do not yet know. Of these few thousand, only 1-2% are used for commercial applications and only a handful are used on a large scale.

Enzymes of the future

A well-known group of enzymes are the hydrolases which act as cleaning agents in detergents. Other well-known, common enzymes are oxidative enzymes. These can initiate many kinds of chemical reactions and they are expected to be the enzymes of the future. The aeration of bread, for example, can be changed by adding certain oxidative enzymes to the dough; other enzymes determine the bitterness of chocolate.

Certain oxidative enzymes are responsible for the undesired brown coloring of fruit and vegetable products, juices and wine. Van Berkel: "Sulphite is the well-known additive which helps prevent these aging processes. But this chemical also causes headaches and other health problems. The challenge is therefore to find new 'healthy' enzyme control mechanisms which prevent the oxidation reactions in foodstuffs. In a European joint venture, we are looking at apple and potato products which must have a fresh appearance for the consumer."

Oxidative enzymes also make an important contribution in the medical world. One of the applications is significant for diabetes patients. Based on a plastic strip containing two different oxidative enzymes, it is possible to read whether their sugar level is good.

Sustainable society

Professor Van Berkel looks at enzymes from the perspective of the enzyme molecule in order to study how it works. 'This is a fundamental approach from the base and not from the end result." Van Berkel's research lays a basis for a sustainable society. "Plants produce extremely interesting compounds," according to the new professor, "but often in small quantities. Furthermore, these compounds are difficult to isolate. By simulating the biochemical reactions, we can produce the rare substances in larger quantities so that we can test them to find out: what works and what doesn't'?" In the long term, the knowledge thus generated can be used in industry.

* Note: this is based on the number of genes discovered to be in the human genome

Source: http://www.sciencedaily.com/releases/2011/05/110502092249.htm

Wageningen University and Research Centre. "Enzymes will play a key role in development of sustainable society, expert says." *ScienceDaily*, 20 Jun. 2011. Web. 26 Aug. 2013.

Floating Disks
Additional Resources

Websites:

http://www.wiley.com/college/boyer/0470003790/animations/animations.htm

http://workbench.concord.org/database/browse/concept/Molecular%20Biology/425.html

Videos:

https://www.youtube.com/watch?v=ClAM75PaQ4Y

http://www.youtube.com/watch?v=ok9esggzN18

http://science.howstuffworks.com/life/28733-assignment-discovery-enzyme-catalysts-video.htm

Games:

http://www.glencoe.com/olc_games/game_engine/content/gln_fcsce/fs_nat_06/ch19/

http://sciencereviewgames.com/srg/games/hs.php?id=83

Related Experiments:

http://library.thinkquest.org/28599/experiment_how_do_enzymes_work.htm

http://www.phschool.com/science/biology_place/labbench/lab2/intro.html

http://www.ableweb.org/volumes/vol-6/10-miller.pdf

Floating Disks

Practice MCAS and AP Questions

Practice MCAS Questions:

The following open-response question from the spring 2013 Biology MCAS test probes student understanding of the effects of temperature on enzyme activity.

Sample student responses can be found at:
http://www.doe.mass.edu/mcas/search/answer.aspx?questionid=29504

- BE SURE TO ANSWER AND LABEL ALL PARTS OF THE QUESTION.
- Show all your work (diagrams, tables, or computations) in your Student Answer Booklet.
- If you do the work in your head, explain in writing how you did the work.

Catalase is an enzyme that protects cells from damage by helping convert the toxin hydrogen peroxide (H_2O_2) into water (HO_2) and oxygen (O_2). A student is investigating how different pH values and different temperatures affect catalase activity. The table below shows the student's data.

Catalase Experiment Data					
Test Tube	**Amount of Catalase (drops)**	**Amount of Hydrogen Peroxide (mL)**	**pH of Solution**	**Temperature of Solution (°C)**	**Relative Rate of Reaction**
1	10	3	1	5	no reaction
2	10	3	1	30	no reaction
3	10	3	1	60	no reaction
4	10	3	3	5	very slow reaction
5	10	3	3	30	slow reaction
6	10	3	3	60	no reaction
7	10	3	7	5	slow reaction
8	10	3	7	30	rapid reaction
9	10	3	7	60	no reaction

a. Identify the test tube that **most likely** has physical conditions similar to the conditions in human cells. Explain your answer.

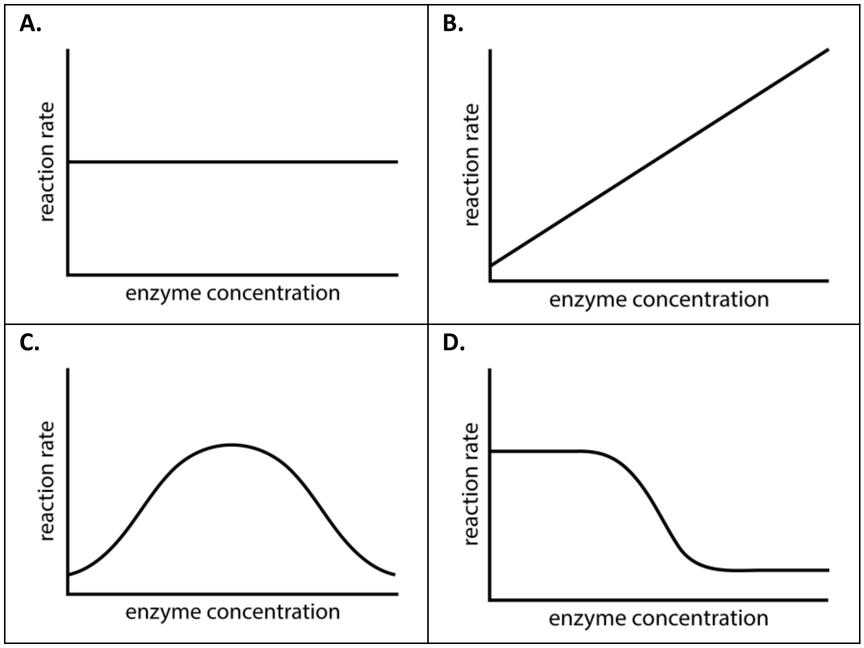
b. Describe how catalase activity changes as pH decreases. Use data from the table to support your answer.

c. Describe how catalase activity changes as temperature increases. Use data from the table to support your answer.

d. Explain why temperature affects catalase activity in the way you described in part C.

The following multiple choice question from the spring 2011 MCAS Biology test probes student understanding of the effects of enzyme concentration on enzyme activity.

A student is investigating how reaction rate changes over a range of enzyme concentrations. The student uses excess substrate(s). Which of the following graphs **best** represents the relationship between enzyme concentration and reaction rate?



Practice AP Exam Questions:

The following free-response question appeared on the 201o AP Biology Exam. Samples of student work and scoring guidelines can be found at:
http://apcentral.collegeboard.com/apc/members/exam/exam_information/219291.html#name10

An experiment was conducted to measure the reaction rate of the human salivary enzyme α-amylase. Ten mL of a concentrated starch solution and 10.0 mL of α–amylase solution were placed in a test tube. The test tube was inverted several times to mix the solution and then incubated at 25°C. The amount of product (maltose) present was measured every 10 minutes for an hour. The results are given in the table below.

Time (minutes)	**Maltose Concentration (μM)**
0	0
10	5.1
20	8.6
30	10.4
40	11.1
50	11.2
60	11.5

(a) **Graph** the data on the axes provided and **calculate** the rate of the reaction for the time period 0 to 30 minutes.
(b) **Explain** why a change in the reaction rate was observed after 30 minutes.
(c) **Draw** and **label** another line on the graph to predict the results if the concentration of α–amylase was doubled. **Explain** your predicted results.
(d) **Identify** TWO environmental factors that can change the rate of an enzyme-mediated reaction. **Discuss** how each of those two factors would affect the reaction rate of an enzyme.

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Standards Alignments

MA Science and Technology/Engineering Standards – High School (2016)

Chemistry

HS-PS1-5. Construct an explanation based on kinetic molecular theory for why varying conditions influence the rate of a chemical reaction or a dissolving process. Design and test ways to slow down or accelerate rates of processes (chemical reactions or dissolving) by altering various conditions

NRC Practices

- Asking questions and defining problems
- Planning and carrying out investigations
- Analyzing data
- Mathematical and computational thinking
- Constructing explanations and designing solutions
- Engaging in argument from evidence
- Obtaining, evaluating, and communicating information

Next Generation Science Standards – High School (2013)

Physical Science

HS-PS1-5. Apply scientific principles and evidence to provide an explanation about the effects of changing the temperature or concentration of the reacting particles on the rate at which a reaction occurs. [

Common Core State Standards Connections:

ELA/Literacy -

RST.9-10.7 Translate quantitative or technical information expressed in words in a text into visual form (e.g., a table or chart) and translate information expressed visually or mathematically (e.g., in an equation) into words.

RST.9-10.8 Assess the extent to which the reasoning and evidence in a text support the author's claim or a recommendation for solving a scientific or technical problem.

RST.11-12.1 Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account.

RST.11-12.7 Integrate and evaluate multiple sources of information presented in diverse formats and media (e.g., quantitative data, video, multimedia) in order to address a question or solve a problem.

RST.11-12.8 Evaluate the hypotheses, data, analysis, and conclusions in a science or technical text, verifying the data when possible and corroborating or challenging conclusions with other sources of information.

RST.11-12.9 Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.

WHST.9-12.1 Write arguments focused on *discipline-specific content.*

WHST.9-12.2 Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.

WHST.9-12.5 Develop and strengthen writing as needed by planning, revising, editing, rewriting, or trying a new approach, focusing on addressing what is most significant for a specific purpose and audience.

WHST.9-12.7 Conduct short as well as more sustained research projects to answer a question (including a self-generated question) or solve a problem; narrow or broaden the inquiry when appropriate; synthesize multiple sources on the subject, demonstrating understanding of the subject under investigation.

WHST.9-12.9 Draw evidence from informational texts to support analysis, reflection, and research.

SL.11-12.5 Make strategic use of digital media (e.g., textual, graphical, audio, visual, and interactive elements) in presentations to enhance understanding of findings, reasoning, and evidence and to add interest.

Mathematics -

MP.2 Reason abstractly and quantitatively.

MP.4 Model with mathematics.

HSF-BF.A.1 Write a function that describes a relationship between two quantities.

HSF-IF.C.7 Graph functions expressed symbolically and show key features of the graph, by hand in simple cases and using technology for more complicated cases.

HSN.Q.A.1 Use units as a way to understand problems and to guide the solution of multi-step problems; choose and interpret units consistently in formulas; choose and interpret the scale and the origin in graphs and data displays.

HSN.Q.A.2 Define appropriate quantities for the purpose of descriptive modeling.

HSN.Q.A.3 Choose a level of accuracy appropriate to limitations on measurement when reporting quantities.

HSS-IC.A.1 Understand statistics as a process for making inferences about population parameters based on a random sample from that population.

HSS-IC.B.6 Evaluate reports based on data.