Activity 2: Determine the Effect of Temperature on the Reaction Rate

Temperature can affect the speed of the reaction. Heat can speed up the movement of the substrate and enzyme molecules, which would increase the number of collisions and therefore speed up the reaction. However, at some point, the forces that allow the enzyme to maintain its proper shape will be broken, changing the shape of the enzyme. The point at which an enzyme changes shape (becomes denatured) will depend on the particular properties of that enzyme. Some enzymes are stable at temperatures close to boiling, whereas others are denatured at room temperature. Most enzymes, however, function best at moderate temperatures (20–40°C). Usually the environment in which the enzyme functions in nature can be a good predictor of the conditions at which it works best in the laboratory. For instance, enzymes produced by bacteria living in hot springs or compost piles can still function at a high temperature, while enzymes produced by bacteria living in arctic ice can function at low temperatures (Groudieva 2004).

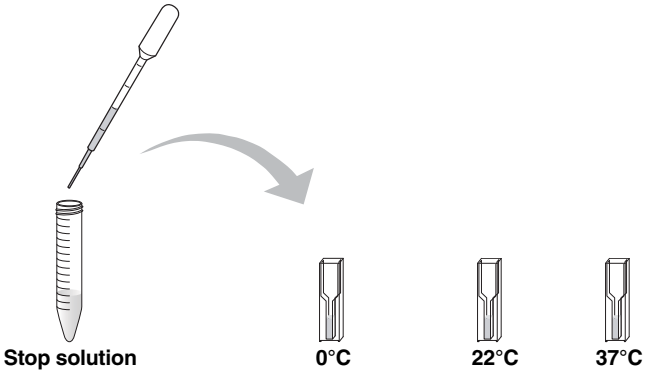
Student Workstation	Quantity	(✔)
1.5 mM substrate	1	❒
Enzyme	1	❒
Stop solution	1	❒
DPTPs	4	❒
Colorimetric standards (S1–S5) from Activity 1	1 of each	❒
Microcentrifuge tubes, 1.5 ml	6	❒
Cuvettes	3	❒
Marker	1	❒
Beaker with ice water	1	❒
Beaker with 37°C water	1	❒
Thermometer	1	❒
Beaker with deionized or distilled water to rinse DPTPs	1	❒
Stopwatch or timer	1	❒

Instructor's Workstation (Optional)	Quantity	(✔)
Spectrophotometer	1	❒

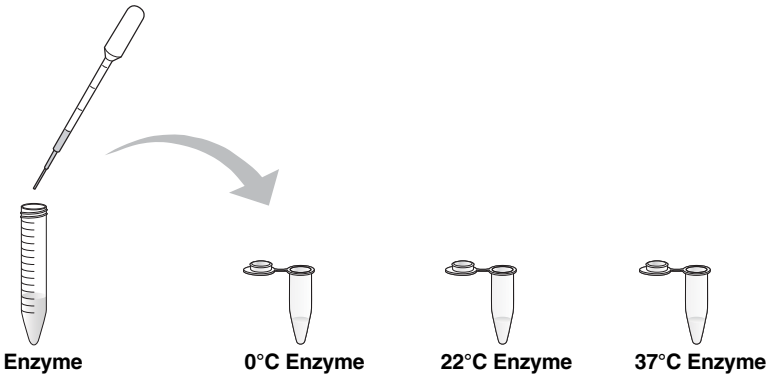
Protocol

1. Label your cuvettes "0°C", "22°C", and "37°C". Only label on the upper part of the cuvette face.

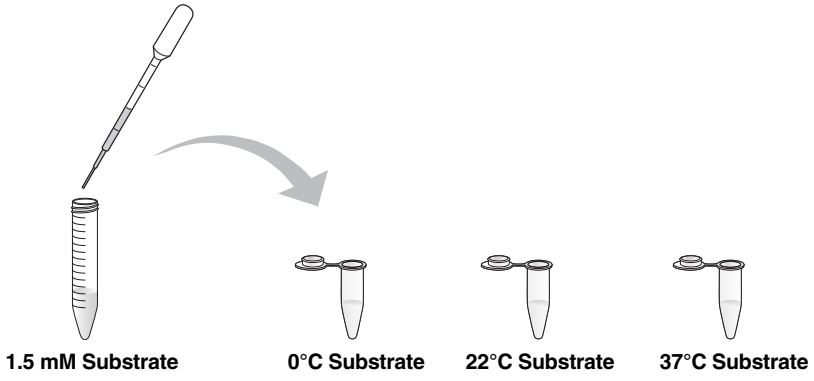
2. Using a clean DPTP, pipet 500 µl of stop solution into each cuvette. Wash the DPTP out thoroughly with water and save.



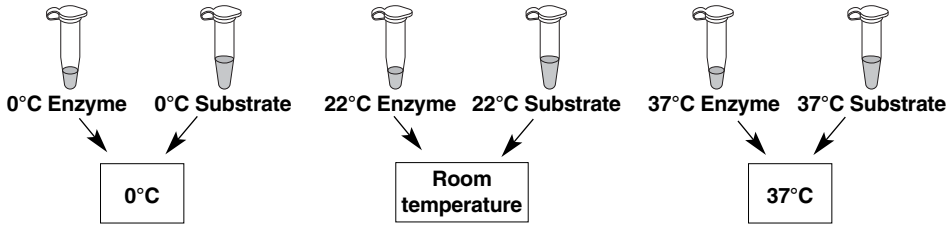
3. Label three 1.5 ml microcentrifuge tubes with “0°C Enzyme”, “22°C Enzyme”, and “37°C Enzyme”. Using a clean DPTP, pipet 250 µl of enzyme into each microcentrifuge tube. Rinse out the DPTP thoroughly with water.



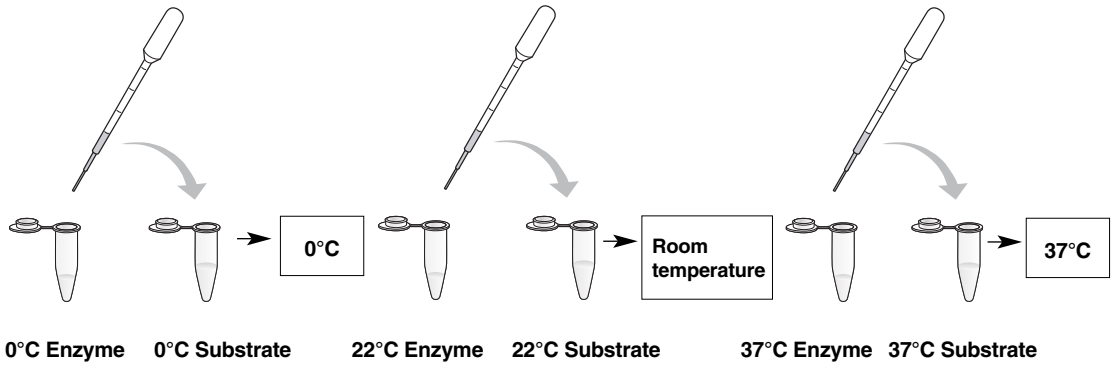
4. Label three 1.5 ml microcentrifuge tubes with “0°C Substrate”, “22°C Substrate”, and “37°C Substrate”. Using a clean DPTP, pipet 500 µl of the 1.5 mM substrate into each microcentrifuge tube. Rinse out the DPTP thoroughly with water.



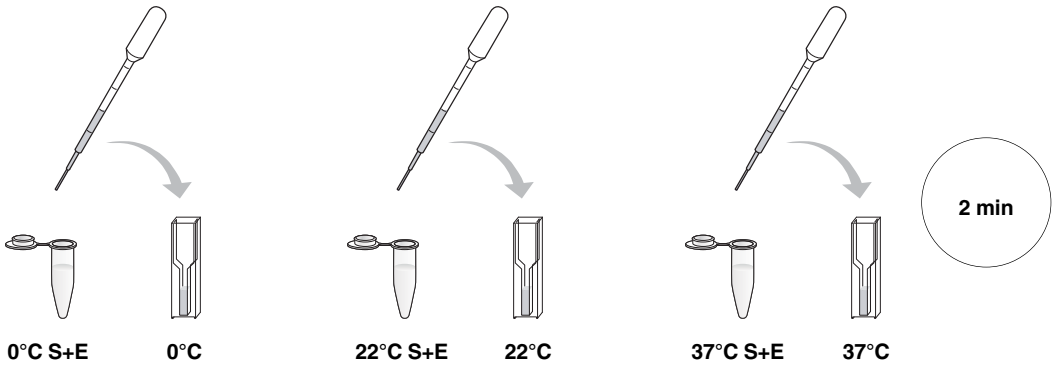
5. Place the microcentrifuge tubes labeled “0°C Enzyme” and “0°C Substrate” in the ice cup. Place the microcentrifuge tubes labeled “22°C Enzyme” and “22°C Substrate” on your lab bench. Place the microcentrifuge tubes labeled “37°C Enzyme” and “37°C Substrate” in the beaker of warm water at 37°C. Allow the tubes to equilibrate to their respective temperatures for at least 5 minutes.



6. Have a stopwatch ready. Using a clean DPTP, pipet the 250 µl of enzyme from the tube labeled “0°C Enzyme” into the tube labeled “0°C Substrate”, and then place the tube now containing your enzyme and substrate mix back on ice. Use the same DPTP to combine the room temperature enzyme and substrate solutions, and place that tube back on the lab bench. Using the same DPTP, combine your 37°C substrate and enzyme solutions, and put that tube back into the water bath. Start your stopwatch.



7. After 2 minutes, use a clean DPTP for each temperature reaction to transfer 500 µl of your reactions to the appropriately labeled cuvettes containing stop solution. Allow all solutions in the cuvettes to reach room temperature for approximately 5 minutes.



8. After all of your samples have been analyzed, rinse out the DPTPs and cuvettes used in this activity with copious amounts of water and save them for future activities.

Note: Do not discard the unused stock solutions or cuvettes containing standards. They will be used for the next activity.

Qualitative Analysis of the Amount of Product Formed at Different Temperatures

1. You should have five cuvettes of standards labeled S1–S5 at your lab bench. Take your 0°C, 22°C, and 37°C cuvette samples, and make a note of the standard that best matches each temperature reaction cuvette. Write down the concentration of that standard (from Table 5 into Table 6) for each of your different temperature reactions.

Table 5. *p*-Nitrophenol standards.

Standard	Amount of *p*-Nitrophenol (nmol*)
S1	0
S2	12.5
S3	25
S4	50
S5	100

*1 nmol = 1 nanomole = 1 x 10^{-9} mol = 0.000000001 mol

Table 6. Determination of *p*-nitrophenol produced at three different temperatures based on *p*-nitrophenol standards.

Temperature	Standard That Is Most Similar	Amount of *p*-Nitrophenol Produced (nmol)
0°C		
~22°C (room temperature)		
37°C		

2. If you do not have a spectrophotometer, please skip ahead to Analysis of Results.

Quantitative Analysis of the Amount of Product Formed at Different Temperatures

1. Blank your spectrophotometer with the S1 standard at 410 nm and then measure the absorbance values for your three cuvettes. Record the absorbance values in Table 7.

2. Following the same protocol used in Activity 1, convert the amount of product you have from units of absorbance measured on the spectrophotometer to nmol, and fill these values in the third column of Table 7.

Table 7. Determination of *p*-nitrophenol produced at three different temperatures based on a standard curve (similar to the one shown in Figure 8).

Temperature	Absorbance at 410 nm	Amount of *p*-Nitrophenol Produced (nmol)
0°C		
~22°C (room temperature)		
37°C		

Analysis of Results

1. Calculate the initial rate of reaction at each of the three different temperatures. Since you only measured the amount of *p*-nitrophenol at one time point (2 minutes), assume that the amount of *p*-nitrophenol at 0 minutes is 0 nmol.

Example: After 2 minutes, the 37°C sample gave an absorbance reading at 410 nm of 0.35, which looked most similar to standard S3. Qualitatively (using the standards), this means that you have ~25 nmol of *p*-nitrophenol. Quantitatively, you would use the standard curve you generated in Activity 1 to determine the amount of p-nitrophenol with an absorbance of 0.35 corresponds to ~22 nmol.

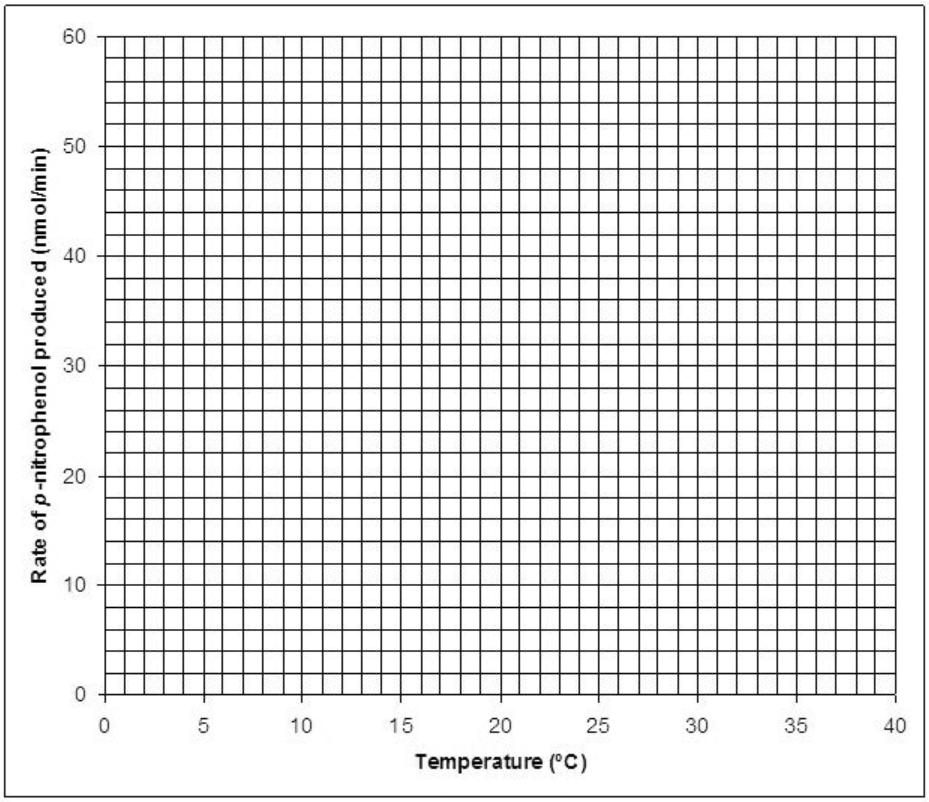
The initial rate for the qualitative data = (25 nmol – 0 nmol)/(2 min – 0 min) = 12.5 nmol/min.

The initial rate for the quantitative data = (22 nmol – 0 nmol)/(2 min – 0 min) = 11 nmol/min.

Perform these calculations for your data

Initial rate of product formation at 0°C =_____nmol/min
Initial rate of product formation at 22°C (room temp) =_____nmol/min
Initial rate of product formation at 37°C =_____nmol/min

2. Plot the effect of temperature on the rate of the enzymatic reaction.



Actvitiy 2 Analysis Questions

1. How can you determine the initial rate of the reaction for each temperature?

2. At what temperature do you think this enzyme works best? How did you come up with your answer?

3. Why do chemical reactions occur faster at higher temperatures?

4. Why do chemical reactions occur more slowly at low temperatures?

5. Why do most enzymatic reactions slow down at extremely high temperatures?

6. If you were a scientist who wanted to use this enzyme to produce glucose, at what temperature should you run the reaction?

7. In what type of environment might an organism that produces this enzyme live? Explain your reasoning.

Challenge questions

1. What types of bonds within the tertiary structure of an enzyme will break at high temperatures? Which ones will not break?

2. Covalent bonds between R groups occur between which amino acids?

3. What would be a disadvantage of using the highest temperature that yields the fastest rate of product formation?