

Quick Guide

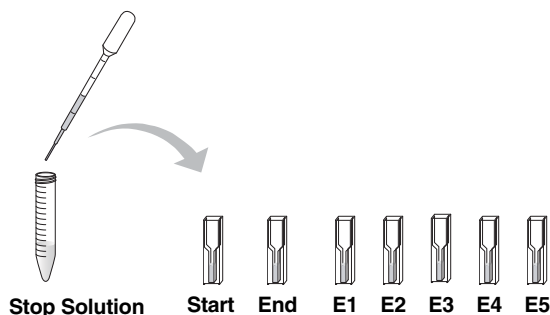
Activity 1: Determine the Reaction Rate in the Presence or Absence of an Enzyme

1. Find your 15 ml conical tubes labeled "Stop Solution", "1.5 mM Substrate", "Enzyme" and "Buffer". Write your initials on each tube.
2. Label five cuvettes E1–E5.



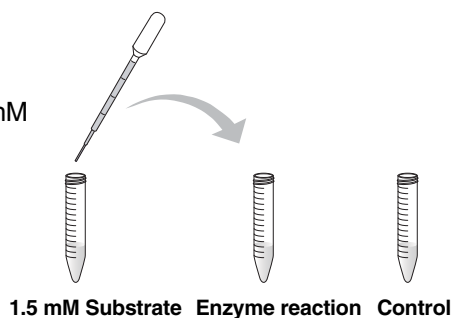
3. Label the two remaining cuvettes "Start" and "End".

4. Using a clean DPTP, pipet 500 μ l of stop solution into each labeled cuvette. Rinse the DPTP well with water.



5. Label one empty 15 ml conical tube "Enzyme Reaction" and the other "Control".

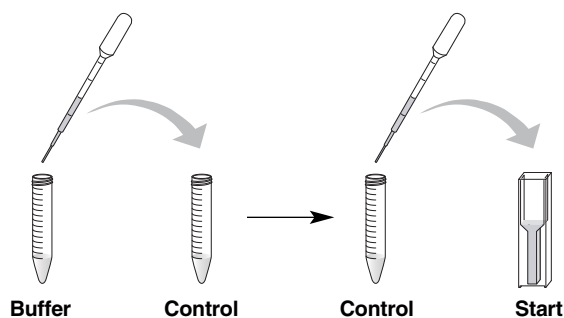
6. Using a clean DPTP, pipet 2 ml of 1.5 mM substrate into the 15 ml conical tube labeled "Enzyme Reaction". Use the same DPTP and pipet 1 ml of 1.5 mM substrate into the conical tube labeled "Control". Rinse the DPTP well with water.



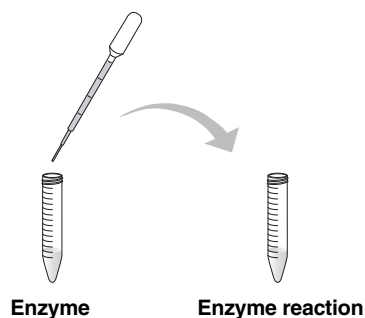
7. Label one DPTP "E" for enzyme and the other "C" for control. Only use the DPTP labeled "E" for the enzyme reaction tube and the DPTP labeled "C" for the control reaction tube.

Read and understand steps 8–11 fully before proceeding. These steps are time sensitive!

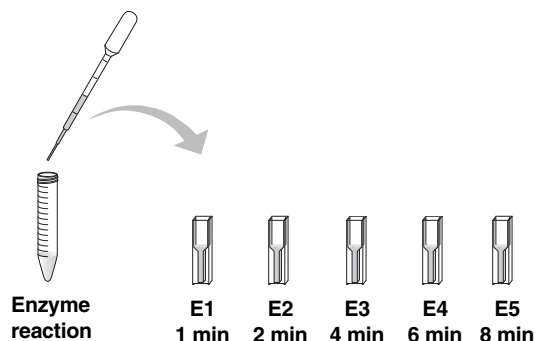
8. Using the DPTP labeled “C”, pipet 500 μ l of buffer into the 15 ml conical tube labeled “Control” and gently mix. Once you have mixed the buffer with the substrate, remove 500 μ l of this solution and add it to your cuvette labeled “Start”.



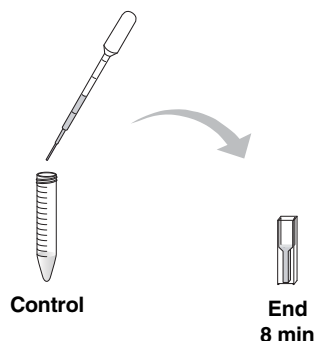
9. Using the DPTP labeled “E”, pipet 1 ml of enzyme into the 15 ml conical tube labeled “Enzyme Reaction”. Gently mix, then **START YOUR TIMER**.



10. At the times indicated, use the DPTP labeled “E” to remove 500 μ l of the solution from the “Enzyme Reaction” tube and add it to the appropriately labeled cuvette containing the stop solution.



11. After all the enzyme samples have been collected, use the DPTP labeled “C” to remove 500 μ l of the solution from the “Control” reaction tube and add it to the cuvette labeled “End”.



12. Proceed with the analysis of your samples. After you have finished your analysis, rinse out your reaction (conical) tubes, cuvettes, and DPTPs with copious water and save them for later activities.

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

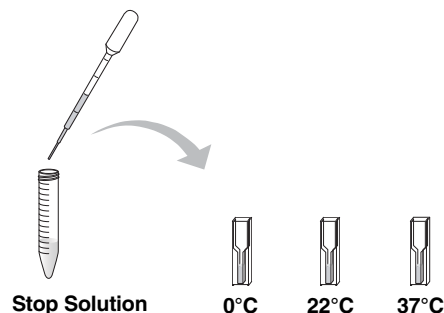
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Activity 2: Determine the Effect of Temperature on the Reaction Rate

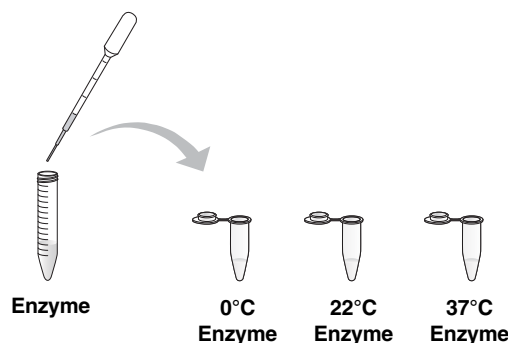
1. Label your cuvettes "0°C", "22°C", and "37°C".



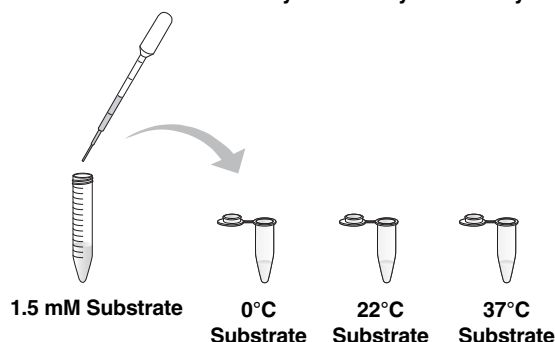
2. Using a clean DPTP, pipet 500 µl of stop solution into each cuvette. Wash the DPTP out thoroughly with water.



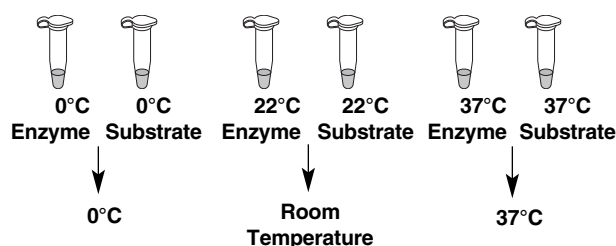
3. Label three 1.5 ml microcentrifuge tubes "0°C Enzyme", "22°C Enzyme", and "37°C Enzyme". Using a clean DPTP, pipet 250 µl of enzyme into each microcentrifuge tube. Rinse out the DPTP thoroughly with water.



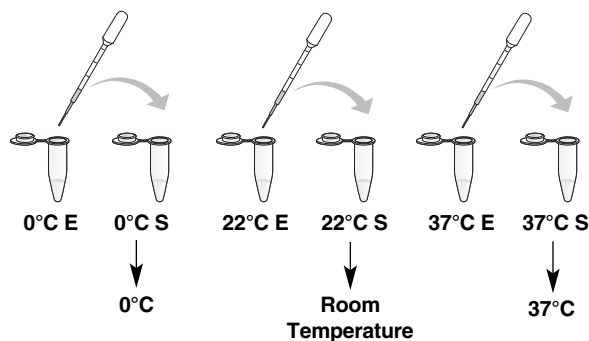
4. Label three 1.5 ml microcentrifuge tubes "0°C Substrate", "22°C Substrate", and "37°C Substrate". Using a clean DPTP, pipet 500 µl of 1.5 mM substrate into each microcentrifuge tube. Rinse out the DPTP thoroughly with water.



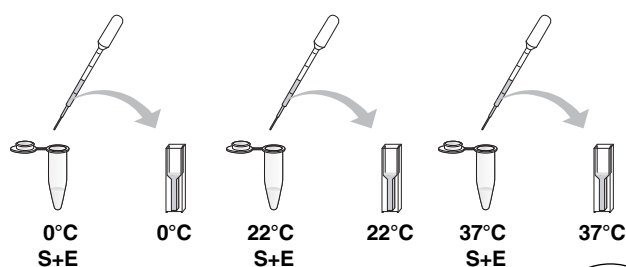
5. Place the tubes labeled "0°C Enzyme" and "0°C Substrate" in the ice cup. Place the tubes labeled "22°C Enzyme" and "22°C Substrate" on your lab bench. Place the tubes labeled "37°C Enzyme" and "37°C Substrate" in the beaker of warm water at 37°C. Allow the tubes to equilibrate to their respective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



- Have a stopwatch ready. Using a clean DPTP, pipet the 250 μ l of enzyme from the tube labeled "0°C Enzyme" into the tube labeled "0°C Substrate", and place the tube now containing your enzyme and substrate mix back on ice. Add the 22°C enzyme to the 22°C substrate solution, and place that tube back on the lab bench. Add the 37°C substrate to the 37°C enzyme solutions, and put that tube back into the 37°C water bath. **START YOUR TIMER.**



- After 2 minutes, use a clean DPTP for each temperature reaction to transfer 500 μ l of your reaction to the appropriately labeled cuvette containing the stop solution.



2 min

- Proceed with the analysis of your samples. After you have finished your analysis, rinse out the cuvettes and DPTPs with copious water and save them for later activities.

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

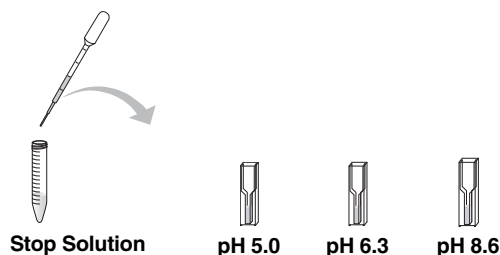
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Activity 3: Determine the effect of pH on the Reaction Rate

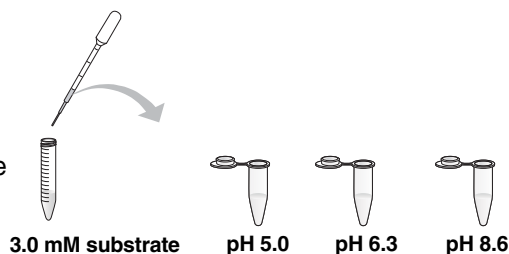
1. Label your cuvettes "pH 5.0", "pH 6.3", and "pH 8.6".



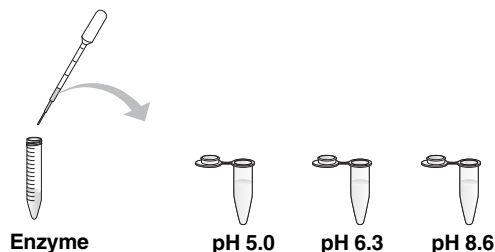
2. Using a clean DPTP, pipet 500 μ l of stop solution into each cuvette. Wash the DPTP out thoroughly with water.



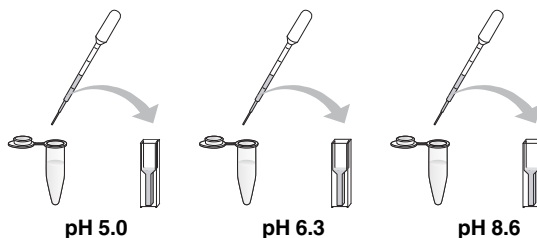
3. Using a clean DPTP, pipet 250 μ l of 3.0 mM substrate into each microcentrifuge tube labeled "pH 5.0", "pH 6.3" and "pH 8.6" by your instructor. Wash the DPTP out thoroughly with water.



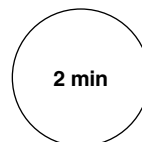
4. Have a stopwatch ready. Using a clean DPTP, add 250 μ l of enzyme to each of the labeled microcentrifuge tubes. **START YOUR TIMER.**



5. After 2 minutes, using a clean DPTP for each pH reaction, transfer 500 μ l of your reaction to the appropriately labeled cuvette containing the stop solution.



6. Proceed with the analysis of your samples. After you have finished your analysis, rinse out the cuvettes and DPTPs with copious water and save them for later activities.

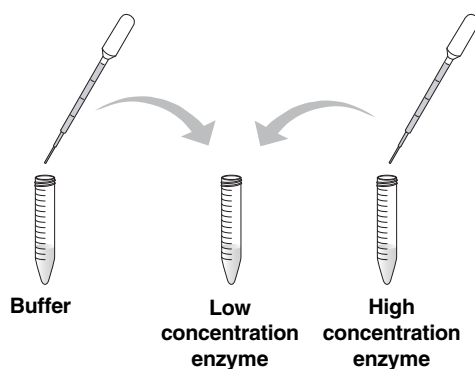


Note: Do not discard unused stock solutions. They will be used for the next activity.

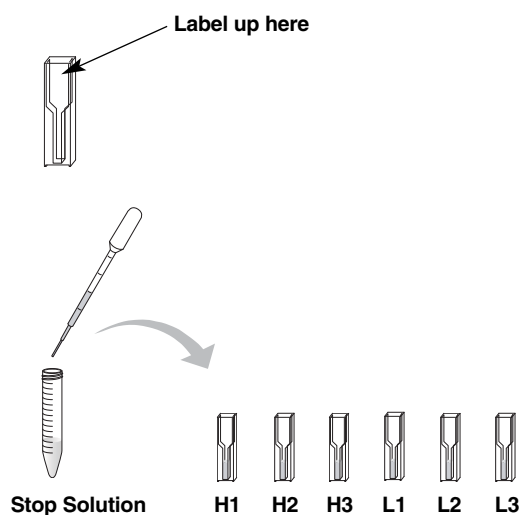
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Activity 4: Determine the Effect of Enzyme Concentration on the Reaction Rate

1. Label one 15 ml conical tube "Low Concentration Enzyme". Using a clean DPTP, pipet 1 ml of buffer into the tube. Wash out the DPTP with water. Pipet 1 ml of high concentration enzyme to your tube labeled "Low Concentration Enzyme" and mix. Wash out the DPTP thoroughly with water.



2. Label three cuvettes "H1–H3" (for high enzyme concentration time points) and the remaining three cuvettes "L1–L3" (for low enzyme concentration time points). Only label on the upper part of the cuvette face.

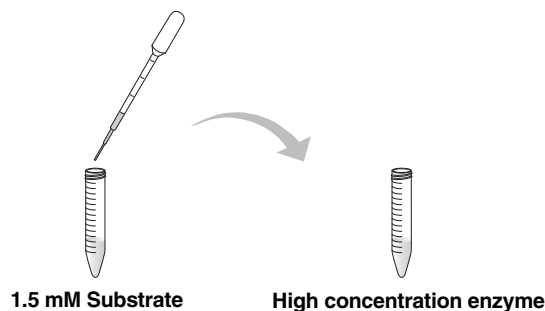


3. Using a clean DPTP, pipet 500 μ l of stop solution into each cuvette. Wash out the DPTP thoroughly with water.

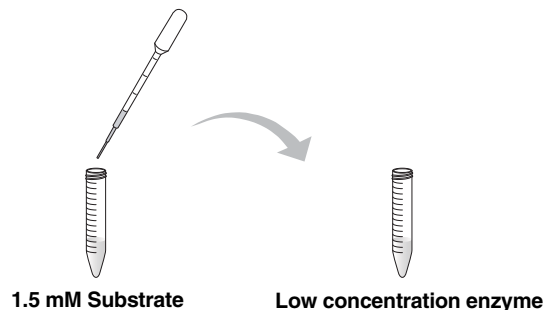
4. Label one clean DPTP with an "H" for high enzyme concentration and a second clean DPTP with an "L" for low enzyme concentration.

Please read steps 5–7 fully before proceeding. These steps are time sensitive!

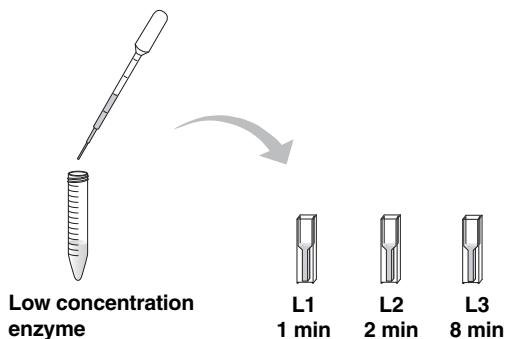
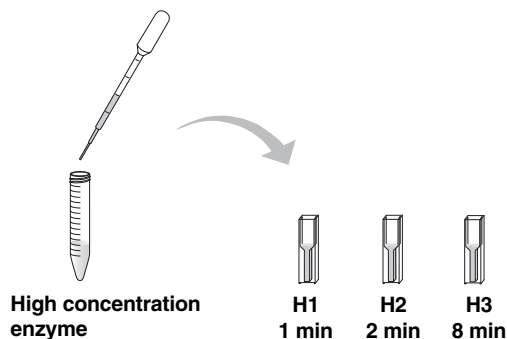
5. Using the DPTP labeled with an “H”, pipet 250 μ l of 1.5 mM substrate into your 15 ml conical tube containing enzyme labeled “High Concentration Enzyme”.



6. Using the DPTP labeled with an “L”, pipet 250 μ l of 1.5 mM substrate into your 15 ml conical tube containing enzyme labeled “Low Concentration Enzyme”. **START YOUR TIMER.**



7. At the times indicated, use the correctly labeled DPTP to remove 500 μ l from the 15 ml conical tubes labeled “High Concentration Enzyme” and “Low Concentration Enzyme”, and add it to the appropriately labeled cuvette that already contains the stop solution.



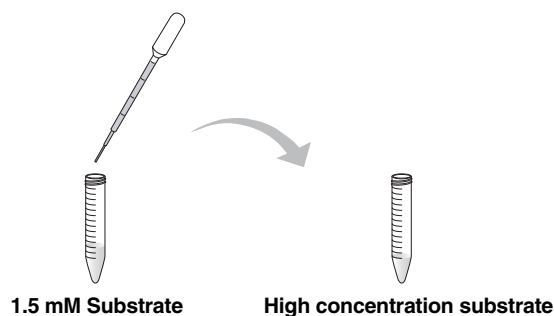
8. Proceed with the analysis of your samples. After you have finished your analysis, rinse out reaction tubes, cuvettes, and DPTPs with copious water and save them for later activities.

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

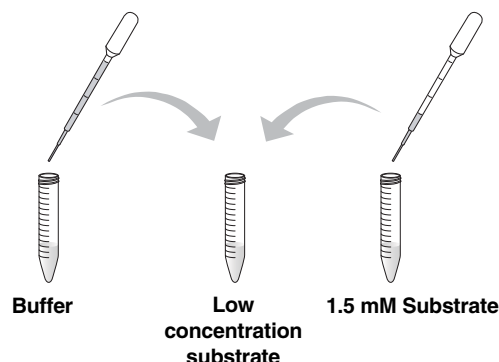
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Activity 5: Determine the Effect of Substrate Concentration on the Reaction Rate

1. Label one clean 15 ml conical tube "Low Concentration Substrate" and one clean 15 ml conical tube "High Concentration Substrate".
2. Using a clean DPTP, pipet 1.5 ml of 1.5 mM substrate into the 15 ml conical tube labeled "High Concentration Substrate". Rinse the DPTP thoroughly with clean water.



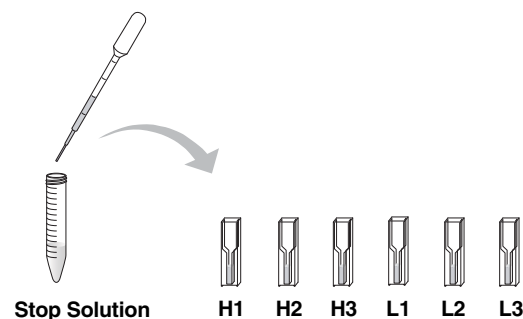
3. Using a clean DPTP, pipet 1.25 ml of buffer into the 15 ml conical tube labeled "Low Concentration Substrate". Rinse the DPTP thoroughly with water and then pipet 250 μ l of 1.5 mM substrate into the 15 ml conical tube labeled "Low Concentration Substrate" and mix. Rinse the DPTP thoroughly with water.



4. Label your cuvettes "H1–H3" (for high substrate concentration time points) and "L1–L3" (for low substrate concentration time points). Only label on the upper part of the cuvette face.

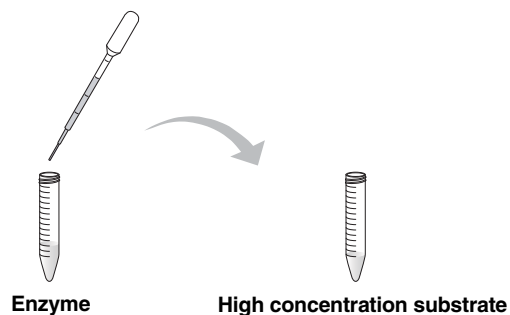


5. Using a clean DPTP, pipet 500 μ l of stop solution into each cuvette. Rinse the DPTP thoroughly with water.
6. Label one DPTP as "H" for high substrate concentration and a second DPTP as "L" for low substrate concentration.

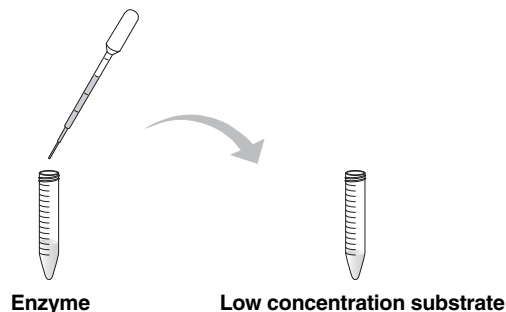


Please read and understand steps 7–9 fully before proceeding. These steps are time sensitive!

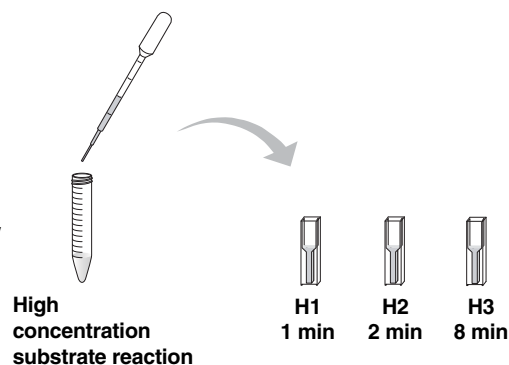
7. Using a clean DPTP, pipet 750 μ l of enzyme into your 15 ml conical tube of substrate labeled “High Concentration Substrate”.



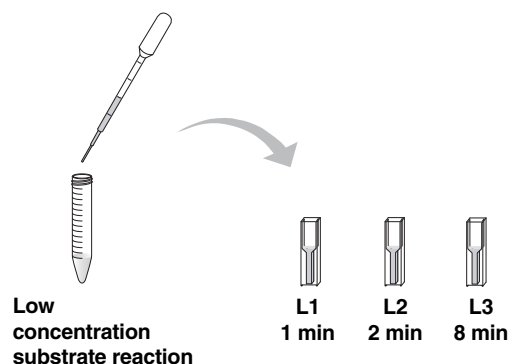
8. Using a clean DPTP, pipet 750 μ l of enzyme into your 15 ml conical tube of substrate labeled “Low Concentration Substrate”. **START YOUR TIMER.**



9. At the times indicated, use the correctly labeled DPTP to remove 500 μ l from the 15 ml centrifuge reaction tubes labeled “High Concentration Substrate” and “Low Concentration Substrate” and add it to the appropriately labeled cuvette that contains the stop solution.



10. Proceed with the analysis of your samples. After you have finished your analysis, rinse out the reaction tubes, cuvettes, and DPTPs with copious water and save them for later activities.



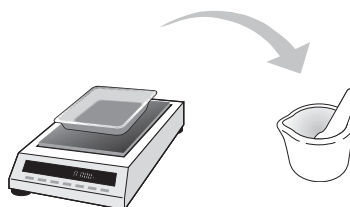
Note: Do not discard unused stock solutions. They will be used for the next activity.

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Activity 6: Test the Ability of Mushroom Extracts to Increase the Reaction Rate

1. Write down the name of your mushroom

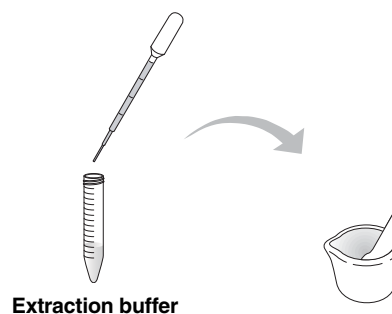
2. Weigh out approximately 1 gram of mushroom and put it into a mortar.



3. Add 2 ml of extraction buffer for every gram of mushroom into the mortar. To calculate the amount of extraction buffer you need, multiply the weight (in grams) of the mushroom by 2 and add that many milliliters.

Weight of mushroom ____ g x 2 = ____ ml

4. Using a pestle, grind your mushroom to produce a slurry.

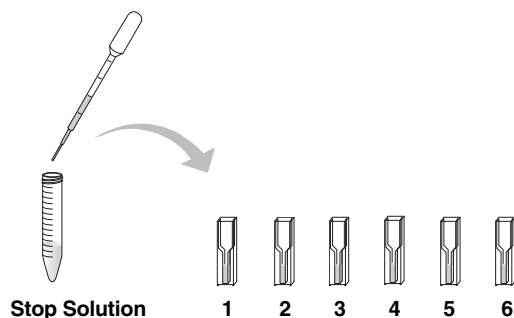


5. Strain the solid particles out of your slurry using a piece of filter paper or cheese cloth into a 1.5 ml microcentrifuge tube. Alternatively, if you have a centrifuge, scoop the slurry into a 1.5 ml microcentrifuge tube and then pellet the solid particles by spinning at top speed for 2 minutes. **Note:** You will need at least 250 μ l of extract to perform the enzymatic reaction.

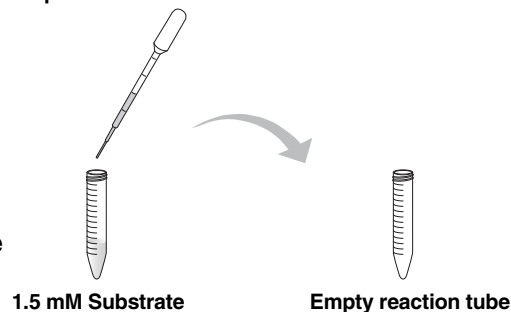
6. Label your cuvettes "1–6". Only label on the upper part of the cuvette face.



7. Using a clean DPTP, pipet 500 μ l of stop solution into each cuvette. Rinse out the DPTP thoroughly with water.

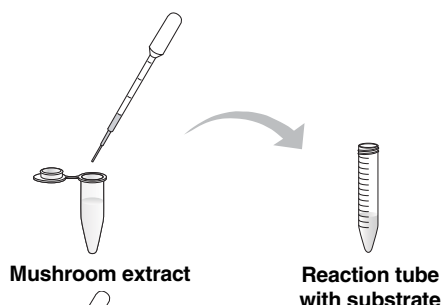


8. Label a 15 ml conical tube with the type of mushroom you are using and then using a clean DPTP, pipet 3 ml of substrate into the tube.

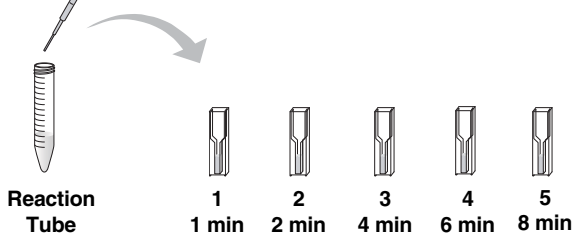


Please read and understand steps 9–10 fully before proceeding. These steps are time sensitive!

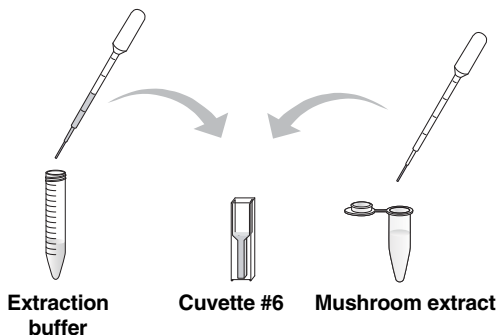
9. Using a clean DPTP, pipet 250 μ l of your enzyme extract into your 15 ml conical tube of substrate. **START YOUR TIMER.**



10. At the times indicated, remove 500 μ l of mushroom extract/substrate mixture from the reaction tube, and add it to the appropriately labeled cuvette that already contains the stop solution.



11. Using a clean DPTP, add 500 μ l of extraction buffer to cuvette #6. Clean the DPTP and then add one drop of mushroom extract. This will serve as the “blank” for this experiment.



12. Proceed with the analysis of your samples. After you have finished your analysis, rinse out the reaction tubes, cuvettes, and DPTPs with copious water and save them for later activities.

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



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