Biofuel Enzyme Reactions Kit for AP Biology: A ThINQ!™ Investigation

Quick Guide

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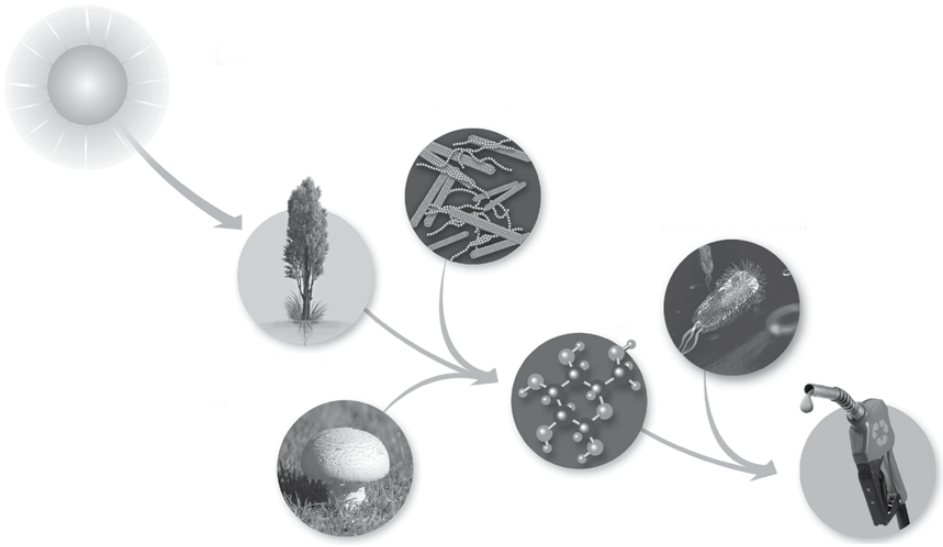

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Quick Guide

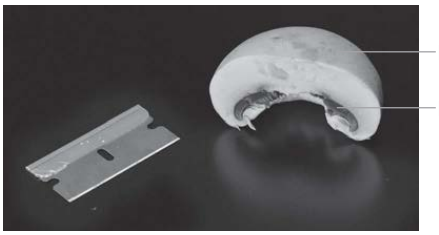
Investigation 1: Determine How Mushroom Extracts Compare in Terms of Cellobiase Activity

1. Write down your mushroom type

   _____

2. Carefully remove the stem of your mushroom if you are using one with a woody stem like white button or shiitake. You will not need to remove the stem if you are using oyster or other soft-stemmed mushrooms.

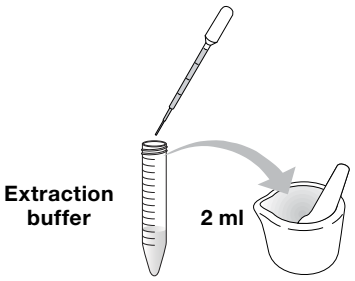
3. Using a razor or knife, cut through the center of the cap of the mushroom. For mushrooms that do not have a distinguishable cap, cut through the meatiest section of the mushroom.



4. Using a cutting implement such as a razor cut out approximately 1 g (1,000 mg) of the internal flesh of the cap. Be careful to avoid including skin and gills in your sample.

5. Weigh out your sample and place it in a mortar: _____ mg

6. Add 2 ml of extraction buffer to the mortar for every gram of mushroom.

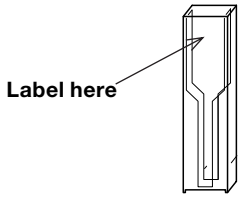


7. Using a pestle, grind your mushroom to produce a slurry — a semiliquid mixture.

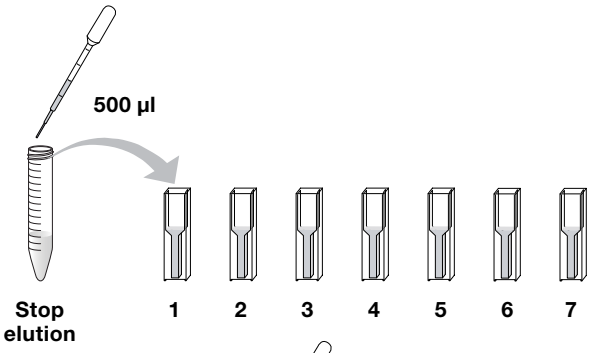
8. Strain the solid particles out of your slurry. If you have a centrifuge, scoop the slurry into a 1.5 ml microcentrifuge tube and then pellet the solid particles by spinning at top speed for 2 min. If you do not have a centrifuge, use a piece of cheesecloth to squeeze the extract into a 1.5 ml micro-centrifuge tube. If you use the cheese-cloth method, you will need to use 2 g mushroom and 4 ml of extraction buffer.

Note: You will need at least 250 µl of extract to perform the enzymatic reaction portion of this protocol.

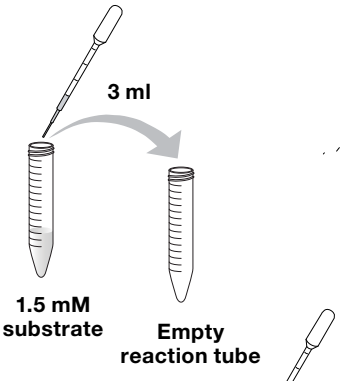
9. Label your cuvettes 1–7. Write only on the upper part of the cuvette face so that your writing won't interfere with spectroscopy.



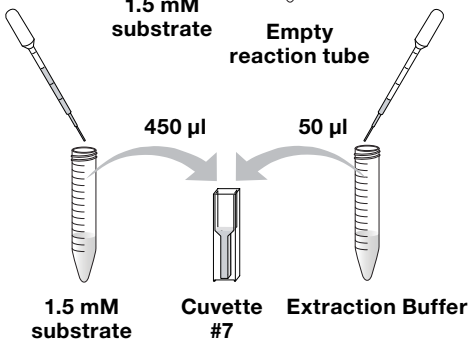
10. Using a clean DPTP, pipet 500 µl of stop solution into cuvettes 1–7. Rinse the DPTP thoroughly with water.



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

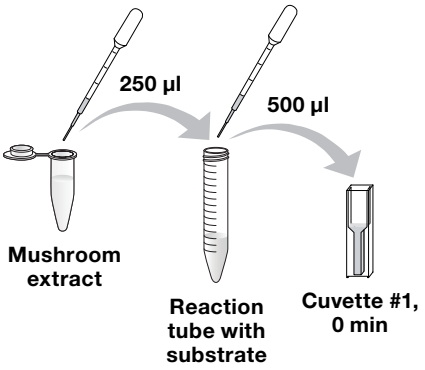


12. Using the same DPTP from the previous step, add 450 µl of substrate and 50 µl of extraction buffer to cuvette #7 and set it aside for analysis later on. This cuvette will act as a control during your investigation because it does not contain mushroom extract.



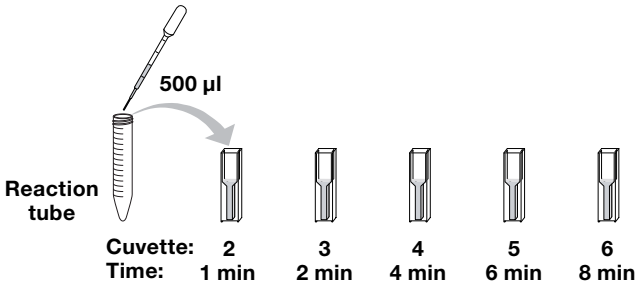
Please read and understand steps 13 and 14 fully before proceeding. These steps are time sensitive!

13. Using a clean DPTP, pipet 250 µl of your mushroom extract into the 15 ml conical tube containing 3 ml of substrate. This will now be referred to as the reaction tube. Pipet the liquid up and down to mix. Then pipet 500 µl from the 15 ml conical (reaction) tube to cuvette #1. **START YOUR TIMER.**



14. At the times indicated in the table remove 500 µl of mushroom extract/substrate mixture from the reaction tube and add it to the appropriately labeled cuvette that already contains stop solution.

Time	*Cuvette*
0 min	1
1 min	2
2 min	3
4 min	4
6 min	5
8 min	6



15. Rinse all DPTPs with copious amounts of water and save them for later investigations. After you have finished your analysis, rinse your reaction (conical) tubes and cuvettes with copious amounts of water and save them for later investigations.

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

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