Bio-Rad Explorer™

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

Planning Guide

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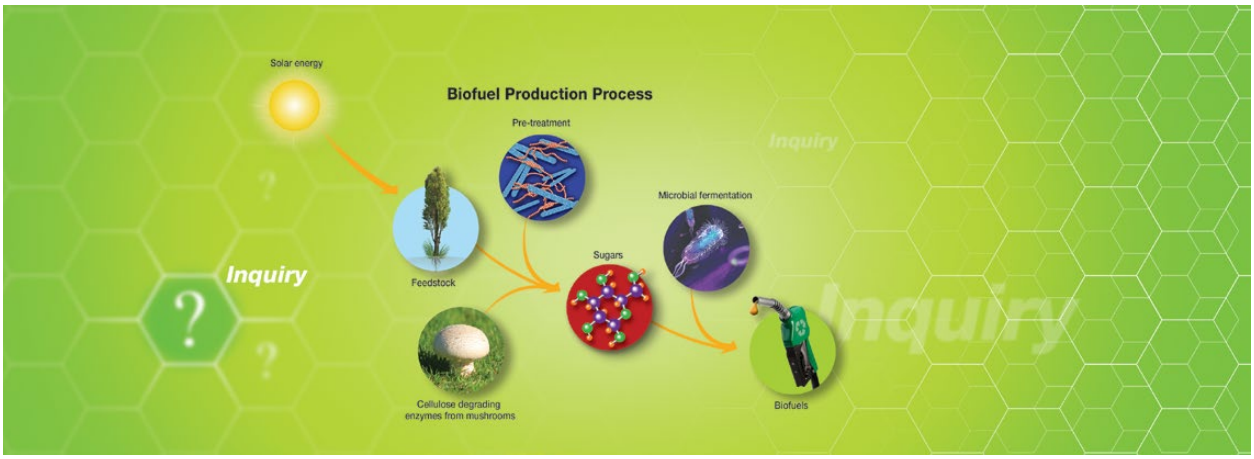
Catalog #17001235EDU, Biofuel Enzyme Reactions Kit for AP Biology



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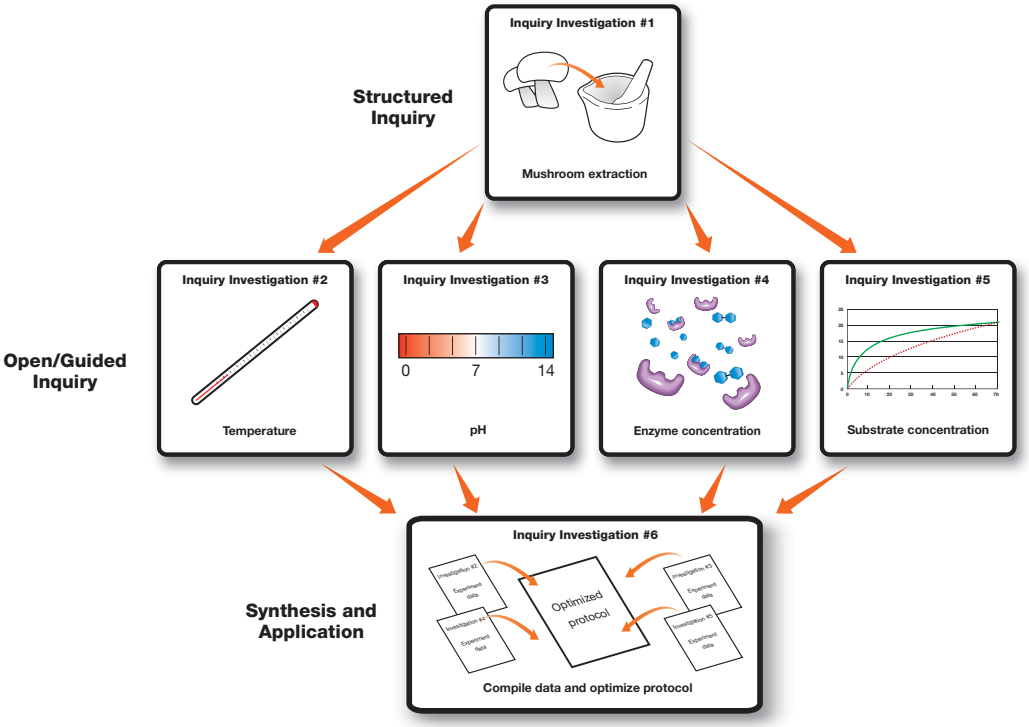
Overview

This planning guide provides an overview of the ThINQ!™ Biofuel Enzyme Reactions Kit for AP Biology — including details of timelines, materials included, and equipment requirements — so that the instructor can prepare curriculum and estimate budgetary requirements in advance. These are estimates based on information available when this guide was created.

The ThINQ! Biofuel Enzyme Reactions Kit for AP Biology (catalog #17001235EDU) is made up of six wet labs that can be completed as structured, guided, and/or open inquiry investigations. A suggested framework is included below, but the kit is flexible and the Instructor's Guide provides support materials to help you adapt the level of inquiry to your classroom needs. ThINQ! kits focus on developing students' ability to navigate the scientific process. The manuals engage and support students in practicing skills such as posing scientific questions and hypotheses, designing experiments, analyzing data, and communicating findings.

The ThINQ! Biofuel Enzyme Reactions Kit tests the ability of an enzyme to increase the conversion rate of a clear substrate to a yellow-colored product. The kit contains sufficient materials for eight student workstations to compare the activity of cellobiase extracted from mushrooms to that of purified cellobiase. Students can test the effects of pH, temperature, and substrate and enzyme concentrations on the rate of enzyme-catalyzed reactions.

Kit Summary



The ThINQ! Biofuel Enzyme Reactions Kit includes one pre-lab activity, six hands-on labs, and a case study. The materials in the kit are sufficient for a class of 32, eight workstations of four students each. We suggest that you conduct Investigation 1 as a structured inquiry lab and Investigations 2–6 as guided or open inquiry labs. But the kit is flexible and will accommodate the needs of your class. Below is an overview of focused goals for each of the activities based on our suggested framework. This may vary, depending on your needs.

Description	Focus Goals
Pre-lab activity: Modeling Enzymatic Reaction	– Make conceptual connections with prior knowledge about enzyme reactions (formative assessment opportunity) – Generate initial model of enzyme reaction
Investigation 1: Compare Cellobiase Activity of Mushroom Extracts	– Practice hands-on laboratory skills – Become familiar with basic enzymatic reaction experiment – Refine enzyme reaction model based on data – Calculate reaction rates based on data
Investigation 2: Determine the Effect of Temperature on the Reaction Rate Investigation 3: Determine the Effect of pH on the Reaction Rate Investigation 4: Determine the Effect of Enzyme Concentration on the Reaction Rate Investigation 5: Determine the Effect of Substrate Concentration on the Reaction Rate	– Engage in the scientific process: – Observe phenomena – Ask scientific questions – Design experiments and protocols – Collect and analyze data – Discuss and communicate findings
Investigation 6: Combine Results and Test an Optimized Protocol	– Synthesize cumulative knowledge and integrate into a higher level investigation
Case Study	– Apply knowledge to real-life science problems

Classic or ThINQ! Kits?

The Biofuel Enzyme Kit is available in two versions. Below is a table that highlights the key features of each.

Description	ThINQ! Biofuel Kit (17001235EDU)	Classic Biofuel Kit (1665035EDU)
Standards alignment	AP Biology	General Biology, Biotechnology, CTE
Support in manual	Open, guided, and structured inquiry	Structured inquiry
Central driver	Student-driven; students and teacher are co-investigators	Teacher-driven; teacher provides scaffolding for students
Main learning objectives	Focus on learning the scientific process: creating hypotheses, designing experiments and protocols, discussion and communication, critical thinking, and problem solving	Focus on learning content and practicing lab techniques: executing defined experimental protocol, mastering technical lab skills and becoming knowledgable about enzyme reactions, reaction kinetics, and the effects of pH, temperature, and substrate and enzyme concentrations on enzyme reactions
Lab workflow	Investigation 1 compares reaction rates of mushroom extracts. This initial experiment increases student engagement	Activity 1 compares reaction rates with and without purified enzyme. This initial experiment provides predictable results

ThINQ! Biofuel Enzyme Reactions Kit for AP Biology Components

Catalog #17001235EDU, ThINQ! Biofuel Enzyme Reactions Kit includes materials for eight student workstations.

Kit Components (included)	Quantity	(✓)
Store at 4°C		
Enzyme, cellobiase, 1 ml	1 vial	☐
Substrate, *p*-Nitrophenyl glucopyranoside, 90 mg	1 vial	☐
Standard, *p*-Nitrophenol (1 mM), 4 ml	1 bottle	☐
2x stop solution, 100 ml	1 bottle	☐
10x resuspension buffer, 50 ml	1 bottle	☐
Extraction buffer, 50 ml	1 bottle	☐
Store at room temperature		
Disposable plastic transfer pipets	40	☐
1.5 ml microcentrifuge tubes	90	☐
15 ml conical tubes	50	☐
1.5 ml standard disposable cuvettes	100	☐
Inquiry curriculum, including Instructor's Guide (Student Manual available online)	1	☐

Required Accessories

Required Accessories (not included)	Quantity per Kit	(✓)
Instructor setup and lab equipment		
500 ml bottle for preparing 1x resuspension buffer	1	☐
200 ml bottle for preparing 1x stop solution	1	☐
100 ml bottle for preparing high-concentration enzyme	1	☐
100 ml bottle for preparing low-concentration enzyme	1	☐
100 ml bottle for preparing 3 mM substrate	1	☐
150 ml bottle for preparing 1.5 mM substrate	1	☐
50 ml tubes or bottles for preparing standards	5	☐
Serological pipettor	1	☐
Pipets or graduated cylinders to measure volumes 4–450 ml	As needed	☐
Deionized or distilled water	1,000 ml	☐
Lab tape	1 roll	☐
Balance (for Investigation 1)	1	☐
Parafilm	1 roll	☐
Razor blade (for Investigation 1)	1	☐
Mushrooms	As needed	☐

Parafilm is a trademark of Bemis Company, Inc.

Student Workstation (4 students)	Quantity per Station	(✓)
All Investigations		
Marking pen	1	☐
Timer or stopwatch	1	☐
Investigation 1: Determine How Mushroom Extracts Compare in Terms of Cellobiase Activity		
Mortar and pestle	1	☐
Weigh boats or weigh paper	1	☐
Filter paper, cheesecloth, or strainer	1	☐
Mushroom sample (minimum 1 g)	1	☐
Investigation 2: Determine the Effect of Temperature on the Reaction Rate and Investigation 6: Combine Results and Test an Optimized Protocol		
Ice bath (beaker with 0ºC water)	1	☐
Beaker with 37ºC water	1	☐
Beaker or ice bucket with chipped ice	1	☐
Thermometer	1	☐

Recommended (Optional) Accessories

Catalog #	Description	Quantity
1660553EDU or 1660508EDU	**100–1,000 µl adjustable micropipet**	8
2239350EDU or 2239040EDU	**100–1,000 µl pipet tips**	8 boxes 1 bag
1702525EDU	**SmartSpec™ Plus spectrophotometer**	1
1660504EDU	**Water bath**, 120 V	1
1660562EDU	**Digital dry bath**, 120 V	1
1660501EDU	**Mini incubation oven**, 120 V	1
1660603EDU	**Mini centrifuge**, 120 V	1

Refills Available Separately

Catalog #	Description
1665036EDU	**Biofuel enzyme kit temperature sensitive reagent refill pack** containing enzyme (cellobiase), substrate (*p*-Nitrophenyl glucopyranoside), standard (*p*-Nitrophenol), 2x stop solution, 10x resuspension buffer, extraction buffer
2239955EDU	**Semimicrovolume disposable polystyrene cuvettes**, 1.5 ml
1660475EDU	**Conical centrifuge tubes**, pkg of 50
1660480EDU	**Disposable plastic transfer pipets, non-sterile**, 500
2239480EDU	**1.5 ml EZ Micro™ test tubes**, pkg of 500

Additional Resources

Additional resources can be found at **bio-rad.com/docs/BiofuelAPresources**. These include:

- Student's Manual
- Experimental Design and Planning Worksheet
- Mushroom habitat flashcards
- Science Case Study
- And many more

Timeline for Preparation and Investigations

The timeline will depend on the students' learning level, class length, and whether additional techniques and analyses are performed in addition to the basic protocol. To assist in planning for the laboratory, the following pages provide a timeline that includes teacher preparation (gray boxes), estimated lab times (white boxes), and suggested homework assignments (italicized). The lessons below are based on 50–60 minute periods and can be modified to suit your classroom situation.

Timeline	Tasks	Estimated Duration
3–7 days prior to Lesson 1	**Instructor's Advance Preparation**	
	1. Read Instructor's Guide	2 hr
	2. Print Student Manual	30 min
Lesson 1	**Pre-Lab Activity: Enzyme Reactions Modeling**	
	1. Guide students through modeling enzyme reactions activity. Alternatively, this can be done as homework followed by a 15 min in-class discussion to assess student understanding of the concepts.	50 min
	2. Assign homework: – *Read background materials, pre-lab materials, and protocol for Investigation 1* – *Complete questions 1.1–1.4*	2 min
1–7 days prior to Lesson 2	**Instructor's Advance Preparation for All Investigations**	
	1. Prepare solutions for all investigations (extraction buffer, substrate, stop solution, and standards).	60 min
	2. Label tubes and cuvettes, and then aliquot solutions and standards for all investigations.	60 min
	3. Inventory required equipment and accessories for all investigations.	30 min
1–3 days before Lesson 2	**Instructor's Advance Preparation for Investigation 1**	
	1. Acquire mushrooms.	As needed
Day of Lesson 2	2. Bring solutions to room temperature (60 min at room temperature).	2 min (+60 min)
	3. Set up workstations.	30 min
	4. Turn on spectrophotometer and warm up for 15 min (only if using spectrophotometer).	2 min (+15 min)
Lesson 2	**Investigation 1: Compare Cellobiase Activity of Mushroom Extracts**	
	1. Cut and weigh mushroom sample.	5 min
	2. Extract and separate mushroom samples.	10 min
	3. Label cuvettes and transfer stop solution to cuvettes.	5 min
	4. Set up control cuvette.	3 min
	5. Label conical tube and set up enzyme reaction.	4 min
	6. Transfer samples of the enzyme reaction to cuvettes at different time points.	8 min
	7. Compare time point samples to *p*-Nitrophenol standards, visually or using a spectrophotometer, and record findings.	10 min
	8. Choose Investigation 2, 3, 4, and/or 5 for each workstation.	3 min
	9. Assign homework: – *Investigation 1 data analysis, post-lab questions, and/or wrap-up questions* – *Prereading and questions for Investigations 2–5* – *Use worksheet to design experimental protocol (for students with less experience designing experiments this can be done in an additional 50 min class session to give students peer and teacher support)*	2 min

Timeline	Tasks	Estimated duration
Lesson 3	**Investigations 2–5:**	
	1. Review student experimental designs.	10 min
	2. Make any necessary dilutions (that is, for pH and substrate and enzyme concentration experiments).	5 min
	3. Label cuvettes and transfer stop solution to cuvettes.	5 min
	4. Set up control cuvette.	3 min
	5. Label conical tube and set up enzyme reaction in chosen conditions.	7 min
	6. Transfer samples of the enzyme reaction to cuvettes at different time points.	8 min
	7. Compare time point samples to *p*-Nitrophenol standards, visually or using a spectrophotometer, and record findings.	10 min
	8. Pool data.	3 min
	9. Assign homework:	2 min
	– *Investigations 2–5 data analysis, post-lab questions, and/or wrap-up questions*	
	– *Develop an optimized protocol for Investigation 6*	
	– *Start lab assessment project (for example, lab report, poster, and/or presentation)*	
Day of Lesson 4	**Instructor's Advance Preparation for Investigation 6**	
	1. Bring solutions to room temperature (60 min at room temp).	2 min (+60 min)
	2. Set up required equipment and materials (for example, water baths).	30 min
	3. Set up workstations.	30 min
	4. Turn on spectrophotometer and warm up for 15 min (only if using spectrophotometer).	2 min (+15 min)
Lesson 4	**Investigation 6: Combine Results and Test an Optimized Protocol**	
	1. Review student experimental designs.	10 min
	2. Label cuvettes and transfer stop solution to cuvettes.	5 min
	3. Set up control cuvette.	3 min
	4. Label conical tubes and set up enzyme reaction in chosen conditions.	7 min
	5. Transfer samples of the enzyme reaction to cuvettes at different time points.	8 min
	6. Compare time point samples to *p*-Nitrophenol standards, visually or using a spectrophotometer, and record findings.	10 min
	7. Assign homework:	2 min
	– *Investigation 6 data analysis, post-lab questions, and/or wrap-up questions*	
	– *Work on lab assessment project (for example, lab report, poster, and/or presentation)*	
	– *Preread Science Case Study (optional)*	
At least 1 day prior to Lesson 5	**Instructor's Advance Preparation for Synthesis and Application**	
	1. Download and print a class set of the Science Case Study: Could Washing Your Pills Down with Juice Be Bad Medicine?	10 min
	2. Read the case study and Instructor's Guide to the Case Study.	45 min
Lesson 5	**Science Case Study: Could Washing Your Pills Down with Juice Be Bad Medicine?**	
	1. Part 1, Read background story and information.	20 min
	2. Part 2, Analyze experimental results.	20 min
	3. Part 3, Develop model of enzyme inhibition.	20 min
	4. Assign homework:	2 min
	– *Complete lab assessment project (for example, lab report, poster, and/or presentation)*	
Lesson 6	**Biofuel Enzyme Reactions Lab Assessment**	
	1. Assess student understanding with a poster session, presentation, and/or lab reports. See Appendix F in Instructor Guide for an example of a biofuels debate to use with your students.	50 min

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