Advance Preparation/Comments

1. Suggest to the students that they become familiar with the exercise before coming to class. If students have a home computer or access to a computer on campus they can become familiar with the general operation of the simulations before coming to class.

2. A short introductory presentation with the following elements is often helpful:
   a. Describe the basics of enzymatic hydrolysis, mentioning how the enzyme-substrate interaction puts stress on the chemical bonds within the substrate to aid in the hydrolytic action.
   b. Students need to clearly understand why the different control tubes are necessary. Explain this concept with plenty of examples.
   c. Because enzymes work as well in vitro as they do in vivo, encourage students to apply what they see in the simulation to what must occur in the lumen of the digestive system.
   d. If a demonstration computer screen is available, briefly show students the basic equipment parts.

3. As the lab progresses, ask students questions directing them to think about the logic of the experiment. For example, if a group of students makes the statement: “Amylase digests starch to maltose,” try asking some of the following questions as the opportunity arises:
   • How do you know that the amylase preparation was not contaminated with maltose?
   • How do you know that the buffer was not contaminated with maltose?
   • How do you know that the water was not contaminated with maltose?
   • How do you know that you even started with starch, and that the starch was not contaminated with maltose?

4. Be prepared to help the students answer the more difficult “What if . . .” questions.

Answers to Questions/Experimental Data

Pre-lab Quiz in the Lab Manual

1. catalysts
2. control
3. salivary amylase (students could also put simply amylase)
4. True
5. blue to black
6. trypsin
7. pancreatic lipase
8. True
9. Segmental
Activity 1: Assessing Starch Digestion by Salivary Amylase (pp. PEx-121–PEx-123)

Predict Question 1: Boiling an enzyme should denature the protein and render it inactive. Freezing the enzyme will have no effect on the enzyme activity because it has little to no effect on enzyme structure.

Chart 1: Salivary Amylase Digestion of Starch

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Additives</th>
<th>Incubation condition</th>
<th>IKI test</th>
<th>Benedict’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amylase Starch pH 7.0 buffer</td>
<td>Boil first, then incubate at 37°C for 60 minutes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Amylase Starch pH 7.0 buffer</td>
<td>Freeze first, then incubate at 37°C for 60 minutes</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Amylase Deionized water Starch pH 7.0 buffer</td>
<td>37°C 60 minutes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Deionized water Starch pH 7.0 buffer</td>
<td>37°C 60 minutes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Deionized water Maltose pH 7.0 buffer</td>
<td>37°C 60 minutes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Amylase Starch pH 2.0 buffer</td>
<td>37°C 60 minutes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Amylase Starch pH 9.0 buffer</td>
<td>37°C 60 minutes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Deionized water Maltose pH 7.0 buffer</td>
<td>37°C 60 minutes</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Activity Questions:
1. The enzyme was no longer active after boiling. Heat denatures enzymes. Freezing had no effect on the activity of the enzyme.
2. The purpose of including Tube #3 is to observe the activity of the enzyme at neutral pH. The enzyme is very active at this pH.
3. The optimum pH is obtained by comparing the results from Tubes #3, 7 and 8.
4. The amylase would not be very active in the stomach because the pH is about 2 and amylase was not very active at this pH.

Activity 2: Exploring Amylase Substrate Specificity (pp. PEx-123–PEx-125)

Predict Question 1: Test Tube #3 should not show a positive Benedict’s test because cellulose is not the substrate for amylase.

Chart 2: Enzyme Digestion of Starch and Cellulose

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Additives</th>
<th>Incubation condition</th>
<th>IKI test</th>
<th>Benedict’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amylase Starch pH 7.0 buffer</td>
<td>37°C 60 minutes</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>Amylase Glucose pH 7.0 buffer</td>
<td>37°C 60 minutes</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Amylase Cellulose pH 7.0 buffer</td>
<td>37°C 60 minutes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Deionized water Cellulose pH 7.0 buffer</td>
<td>37°C 60 minutes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Peptidase Starch pH 7.0 buffer</td>
<td>37°C 60 minutes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Bacteria Cellulose pH 7.0 buffer</td>
<td>37°C 60 minutes</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Activity Questions:
1. No, amylase doesn’t use cellulose as a substrate. Starch is the substrate for amylase.
2. The bacteria were able to digest the cellulose because they produce the enzyme, cellulase.
3. The peptidase had no effect on the starch because the substrate for peptidase is peptides and proteins.
4. The smallest subunit that starch can be broken down into is glucose.

Activity 3: Assessing Pepsin Digestion of Protein (pp. PEx-125–PEx-126)
Predict Question 1: It should be pH 2 because pepsin is most active in the stomach.

Chart 3: Pepsin Digestion of Protein

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additives</td>
<td>Pepsin BAPNA pH 2.0 buffer</td>
<td>Pepsin BAPNA pH 2.0 buffer</td>
<td>Pepsin Deionized water BAPNA pH 2.0 buffer</td>
<td>Deionized water BAPNA pH 2.0 buffer</td>
<td>Pepsin BAPNA pH 7.0 buffer</td>
<td>Pepsin BAPNA pH 9.0 buffer</td>
</tr>
<tr>
<td>Incubation condition</td>
<td>Boil first, then incubate at 37°C for 60 minutes</td>
<td>37°C 60 minutes</td>
<td>37°C 60 minutes</td>
<td>37°C 60 minutes</td>
<td>37°C 60 minutes</td>
<td>37°C 60 minutes</td>
</tr>
<tr>
<td>Optical density</td>
<td>0.00</td>
<td>0.40</td>
<td>0.00</td>
<td>0.00</td>
<td>0.03</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Activity Questions:
1. The optimum pH matches the pH secreted by gastric glands. Gastric juice is also about pH 2.
2. Since the pH of the mouth is closer to neutrality, you would expect pepsin to be slightly active but not as active as it is in the stomach at pH 2.
3. The subunit products of digestion are peptides and amino acids.
4. The control tube #4 is present to make certain that the BAPNA is not breaking down due to the low pH.

Activity 4: Assessing Lipase Digestion of Fat (pp. PEx-127–PEx-128)
Predict Question 1: Test tube #1 should have the highest activity because the pH is closest to the pH of the small intestine. Note: Some students might choose pH 9.0 but the intestine is closer to 8.0 and the enzyme is not active at such high alkalinity.

Chart 4: Pancreatic Lipase Digestion of Triglycerides and the Action of Bile

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additives</td>
<td>Lipase Vegetable oil Bile salts pH 7.0 buffer</td>
<td>Lipase Vegetable oil Bile salts pH 7.0 buffer</td>
<td>Lipase Deionized water Bile salts pH 9.0 buffer</td>
<td>Deionized water Vegetable oil Bile salts pH 7.0 buffer</td>
<td>Lipase Vegetable oil Bile salts pH 2.0 buffer</td>
<td>Lipase Vegetable oil Bile salts pH 9.0 buffer</td>
</tr>
<tr>
<td>Incubation condition</td>
<td>37°C 60 minutes</td>
<td>37°C 60 minutes</td>
<td>37°C 60 minutes</td>
<td>37°C 60 minutes</td>
<td>37°C 60 minutes</td>
<td>37°C 60 minutes</td>
</tr>
<tr>
<td>pH</td>
<td>6.21</td>
<td>6.72</td>
<td>9.00</td>
<td>7.00</td>
<td>2.00</td>
<td>8.97</td>
</tr>
</tbody>
</table>

Activity Questions:
1. Lipase activity is measured by a decrease in pH through the release of fatty acids.
2. The pH in tube #5 is pH 2.0. The pH is too low to be able to see if any fatty acids have been released.
3. Pancreatic lipase would be active in the mouth since the pH of the mouth is close to 7.0 and the enzyme is most active at this pH.
4. Fat globules are separated into droplets by bile salts through an emulsification process which is physical not chemical.
**ACTIVITY 1**
**Assessing Starch Digestion by Salivary Amylase**

1. List the substrate and the subunit product of amylase.  
   The substrate of amylase is animal starch and the product is maltose and glucose.

2. What effect did boiling and freezing have on enzyme activity? Why? How well did the results compare with your prediction?  
   The boiling denatured the enzyme and inactivated it as predicted. The freezing has no effect on the enzyme.

3. At what pH was the amylase most active? Describe the significance of this result.  
   Amylase was most active at pH 7.0. This is significant because this is the same pH as the mouth.

4. Briefly describe the need for controls and give an example used in this activity.  
   Controls are necessary to validate the results of the experiment. Tube #5 is an example where the enzyme that tests for contaminating glucose in the starch or the buffer is absent.

5. Describe the significance of using a 37°C incubation temperature to test salivary amylase activity.  
   The 37°C incubation is significant because it is the same temperature as body temperature so it should be ideal for the enzyme.

**ACTIVITY 2**
**Exploring Amylase Substrate Specificity**

1. Describe why the results in tube 1 and tube 2 are the same.  
   In tube #1 the amylase is hydrolyzing the starch to glucose and in tube #2 the glucose is already present in the hydrolyzed form.

2. Describe the result in tube 3. How well did the results compare with your prediction?  
   The correct prediction is “no”. Tube #3 should not be positive for the Benedict’s test because amylase should not digest cellulose.

3. Describe the usual substrate for peptidase.  
   The usual substrate for peptidase is peptides and proteins.

4. Explain how bacteria can aid in digestion.  
   Bacteria can aid in digestion by breaking down cellulose which we do not produce cellulase.
**Activity 3** Assessing Pepsin Digestion of Protein

1. Describe the effect that boiling had on pepsin and how you could tell that it had that effect. *Boiling inactivated the pepsin.*
   
   *This is evidenced by the fact that no activity was seen with Tube #1. However, the enzyme was very active in Tube #2.*

2. Was your prediction correct about the optimal pH for pepsin activity? Discuss the physiological correlation behind your results.
   
   *The correct prediction is pH 2.0. This is because pepsin is most active at the pH of gastric juice which is about pH 2.0.*

3. What do you think would happen if you reduced the incubation time to 30 minutes for tube 5? *If the incubation time were reduced, it is possible that no digestion of protein would be seen since only a small amount is seen.*

**Activity 4** Assessing Lipase Digestion of Fat

1. Explain why you can’t fully test the lipase activity in tube 5. *Measurement of lipase activity uses a decrease in pH. Since the pH in Tube #5 is already very low, it is difficult to tell if fatty acids are released.*

2. Which tube had the highest lipase activity? How well did the results compare with your prediction? Discuss possible reasons why it may or may not have matched. *The correct prediction is Tube #1, pH 7.0, which approximates the pH of the small intestine.*

3. Explain why pancreatic lipase would be active in both the mouth and the intestine. *Since the activity of pancreatic lipase is highest at pH 7.0, the enzyme should be active in the mouth and the pancreas.*

4. Describe the process of bile emulsification of lipids and how it improves lipase activity. *Bile serves to mechanically break up large globules of fat and produce small droplets that effectively increases the surface area of the lipids.*