

# LOOKING INTO LACTASE BIOTECHNOLOGY Lab

*Pharmex  
Pharmaceuticals*

## SCITECH

## STUDENT

## PACKET

### **MEMO**

I commend all of you research team members for your hard work to date. I thought it might help to remind you why it is so important for us to work diligently for the successful development of our Lactex® product. I have enclosed a flier that I picked up at a doctor's office, which includes a **Medical Fact Sheet**. Please share this flier with your team members.

Our newest product, Lactex®, is nearly ready for pre-clinical trials. If the results from these pre-clinical trials are promising, we will request permission from the U.S. Food and Drug Administration (FDA) to begin Phase I clinical trials. Before this, however, we need to verify Lactex®'s ability to break down the disaccharide lactose into glucose and galactose. We will also need to check Lactex®'s ability to function at different temperatures and different pH values.

You are to work in your teams to conduct three experiments. Please submit your final laboratory protocols, which include your results, after completion. Also remember to follow established safety procedures as we do not want to be fined by OSHA (Occupational Safety & Health Administration).

# LOOKING INTO LACTASE

## BIOTECHNOLOGY Laboratory Protocol and Worksheet

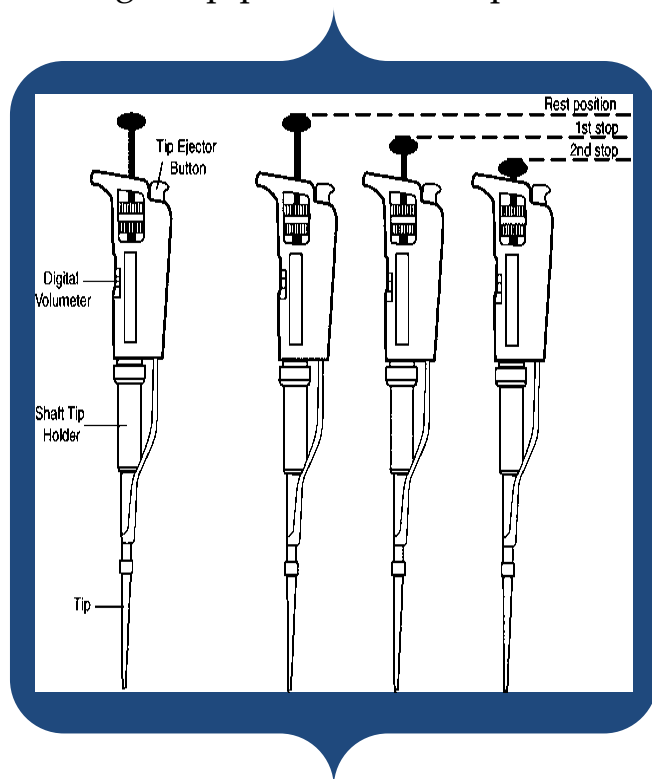
### Micropipette Explanation

1 L = 1,000 mL

1 mL = 1,000  $\mu$ L

#### Micropipette Procedure

1. Adjust the pipette to the desired volume by turning the dial. Do not turn the dial beyond the volume range for the pipette.
2. Firmly press a new tip onto the pipette by inserting the pipette into the tip while the tip is still in the box.
3. Get tip out without touching it with your hands; this is to prevent contamination of the samples.
4. Draw up liquid from the micropipette
  - Depress the plunger to the first stop to measure the desired volume and hold in that position.
  - Holding the pipette vertically, immerse only the very end of the tip into the liquid to be transferred.
  - Slowly release the plunger to draw up the liquid.
  - Wait 1 – 2 seconds to be sure the full volume of sample is drawn up into the tip.
5. Dispense the liquid
  - Place the tip into the container where the liquid is to be released.
  - Slowly depress the plunger to the second stop to blow out all of the liquid in the tip.
  - Remove tip out of liquid.
  - Release plunger carefully.
  - Eject tip into a waste container.

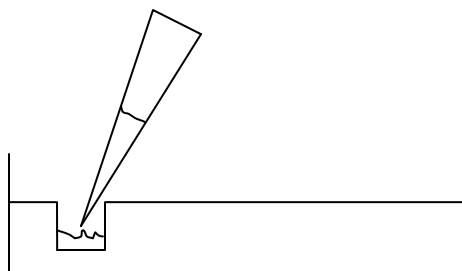


## LOOKING INTO LACTASE

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## Practice Gel Loading Exercise

1. Set the micropipette to 10  $\mu\text{L}$
2. Add a tip on the end of the micropipette.
3. Remove the lid of the practice agarose gel
4. Make sure you can clearly see the wells.
5. Microcentrifuge the practice loading dye.
6. Select a well to pipette the dye into.
7. Draw up 10  $\mu\text{L}$  of loading dye.
8. Lower the tip filled with the dye into a well to be filled.
9. Carefully dispense all of the 10  $\mu\text{L}$  of dye into the well.
10. Examine your practice gel to make sure that the tip did not poke through the bottom of the well or rip between the wells.
11. Repeat steps 1 – 10 until you are comfortable with loading samples onto a gel.



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### **Terminology:**

**Activation Energy:** The minimum amount of energy required to convert a normal stable molecule into a reactive molecule.

**Active Site:** The region of a biologically active protein (an **enzyme**) where catalytic activity takes place and whose shape permits the binding of a specific reactant molecule (the **substrate**).

**Denature:** To modify the structure of a molecule by heat, acid, alkali, or ultraviolet radiation so as to destroy or diminish some of the original properties and especially the specific biological activity.

**Disaccharide:** Any of a class of sugars (such as sucrose and lactose) that on hydrolysis yields two monosaccharide molecules.

**Enzyme:** Biologically active proteins that are produced by living cells and catalyze specific biochemical reactions.

**Hydrolysis:** A chemical process involving the splitting of a bond and the addition of the hydrogen cation and the hydroxide anion of water. In this lab, hydrolysis of a disaccharide yields two monosaccharides.

**Lactase:** An enzyme that breaks down lactose into glucose and galactose and occurs especially in the intestines of young mammals.

**Lactose:** A disaccharide sugar that is present in milk that yields glucose and galactose upon hydrolysis.

**Lactose Intolerance:** Lactose intolerance is an inability to digest and absorb lactose due to a deficiency in the enzyme that breaks down lactose (lactase). This condition, while treatable, results in gastrointestinal symptoms when milk or products containing milk are ingested.

**Lock and Key Model:** A theory for enzyme activity which states that all enzymes and substrates have specified structures (**active sites**). In this analogy, the lock is the enzyme and the key is the substrate. Only the correctly sized key (**substrate**) fits into the key hole (**active site**) of the lock (**enzyme**).

**Monosaccharide:** A sugar not decomposable to simpler sugars by hydrolysis. Glucose, a building block of lactose, is an example of a monosaccharide.

**Organic Catalyst:** A substance (an enzyme) that modifies and increases the rate of a chemical reaction (by lowering the activation energy) without being consumed or permanently altered by the reaction.

**Substrate:** The material or substance on which an enzyme acts.

**Three-dimensional Shape:** Refers to the biologically active shape of a protein that determines its characteristic properties. All enzymes have a specific three-dimensional shape that enables them to fit with their particular substrate (which has a complementary shape) and thereby catalyze reactions.

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## BIOTECHNOLOGY Laboratory Protocol and Worksheet

### Medical Fact Sheet

**What is Lactose Intolerance?** Lactose intolerance is a medical condition that is due to little or no activity of the enzyme lactase, which prevents lactose from being broken down into glucose and galactose. With low lactase activity in the small intestine, undigested lactose is passed into the colon where bacteria ferment the sugar to hydrogen gas and organic acids. Gastrointestinal symptoms of lactose intolerance can include bloating, abdominal pain, cramps, gas, flatulence, and sometimes diarrhea. The symptoms usually are noticed anywhere from 30 minutes to 2 hours after the ingestion of dairy products containing lactose. The severity of symptoms varies by individuals depending upon how much lactose is consumed and whether it is consumed alone or with other foods. The type of dairy products can also affect the severity of symptoms because some dairy products contain more or less lactose. For example, certain hard or aged cheeses like cheddar and Swiss contain less lactose than drinking a glass of milk, and therefore are easier to tolerate. Yogurt, which contains active bacterial cultures, is better tolerated as well because the yogurt contains enzymes that digest lactose.

**What Diseases Are Caused by Lactose Intolerance?** Avoiding dairy products may result in an inadequate dietary intake of calcium, which may lead to various diseases including rickets, osteoporosis, and hypertension. Dairy foods are an excellent source of natural calcium, protein, vitamins A and D and other nutrients. Calcium is especially important for healthy bones and teeth. Milk is the primary source of calcium for children. It is necessary that children who are lactoseintolerant obtain the necessary calcium through some supplemental means.

**What Medical Care is Available?** Forms of medical care for those suffering from the symptoms of lactose intolerance include the manipulation of the diet by carefully eliminating certain milkcontaining products. Another form of medical care is the use of supplemental lactase derived from yeast and other fungal species. These can be in the form of liquid drops or chewable tablets. Individuals can take these prior to the ingestion of lactose-containing foods. There are also lactasesupplemented milk and dairy products now available.

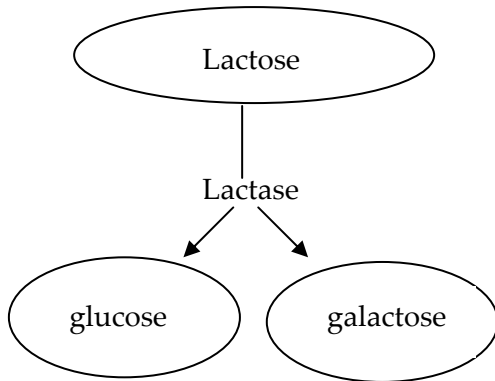
**What Causes Lactose Intolerance?** The gene for the production of lactase is located on chromosome two. However there does not seem to be any difference in the DNA of individuals with or without lactase activity. Rather the differences are in the messenger RNA (mRNA), which may indicate that the primary regulation of this enzyme occurs during translation.

**Who Is Lactose Intolerant?** Both childhood- and adulthood-onset of lactase deficiency are very common and are inherited as autosomal recessive. Those individuals who have lactase activity that persists into adulthood have inherited at least one dominant gene. Patterns of inheritance show racial variability. Northern Europeans have the lowest prevalence of adulthood-onset lactase deficiency at approximately 5%. Central Europeans have a prevalence of about 30%. Southern Europeans, as well as Hispanic and Jewish populations, show a high prevalence of approximately 70% adult-onset of lactase deficiency. While Northern Indians show a 25% prevalence, Southern Indians show a much higher prevalence of 65%. Both Asians and Africans are nearly all (90%) lactose intolerant as adults.

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## BIOTECHNOLOGY Laboratory Protocol and Worksheet

### PART 1: Test for the Resistance of Glucose & Lactase Enzyme Activity



You are a scientist at a company called Pharmex. In order to verify the activity of lactase, it must be tested on different milks which contain different sugars to verify that it only works on lactose. If the enzyme has additional activities which affect other sugars, it may not be approved for use by the FDA. Three milk samples will be tested, cow, soy, and rice. This is a blind test to avoid bias and it will be your job to determine which milk is which and to prove that lactase breaks down only lactose.

Milk	Sugar
Cow	Lactose
Soy	Sucrose
Rice	Glucose

Independent Variable: \_\_\_\_\_

Dependent Variable: \_\_\_\_\_

#### Test unknown milk samples for glucose:


1. Identify the 3 unknown milk samples labeled #1, #2, and #3 at your station.
2. Label three glucose test strips # 1, # 2, and #3.
3. **Do not touch the test pad with your hands.** Close container after you remove strips.
4. Take glucose test strip #1 and dip it into tube #1. Make sure the test pad get really wet.
5. Immediately remove the test strip and lay it down on the paper towel.
6. Repeat for samples #2 and #3.

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7. Keep the strips separated on the paper towels.
8. Wait 3 minutes and record the color of the test strip in Table 1. This gives a base-line amount of glucose before anything has been done to the milk samples.
9. Compare the color which develops on the test strip to the color chart and record the relative amount of glucose in Table 1.
10. Discard the used glucose strips in the waste container.

### **Test unknown samples for lactase enzyme activity**

11. Identify the tube labeled “Lactex®” and mix gently by inversion to get an even distribution of the powder through the solution.
12. **Do not shake vigorously** or produce bubbles as this may damage the enzyme.
13. Using the pipette labeled “Lactex®” add 1 ml of the solution to tubes #1, #2, and #3.
14. **DO NOT TOUCH** tubes with the end of the pipette or your samples will be contaminated. Use proper pipette technique.
15. **Firmly cap tubes #1, #2, #3.** Vortex all tubes for **NO MORE THAN 2 seconds.**
16. Dispose the previously used test strips and remove 3 new strips. Label strips #1, #2, and #3.
17. Take new glucose test strip #1 and dip it into tube #1 (just enough to get the test pad wet).
18. Immediately remove the test strip and lay it down on the paper towel.
19. Repeat for samples #2 and #3. Keep the strips separated on the paper towels.
20. **Wait 3 minutes.** 
21. While you are waiting, label 3 new glucose test strips as #1, #2, #3.
22. Record the color of the test strip in **Table 1.** This shows any breakdown of the sugar to produce glucose.

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### **BIOTECHNOLOGY Laboratory Protocol and Worksheet**

23. Compare the color which develops on the test strip to the color chart on the test strip bag and record the relative amount of glucose in **Table 1**.
24. Identify the milk contained in samples #1-3 in **Table 1** below. Use the test results and the chart provided below as a guide.

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## BIOTECHNOLOGY Laboratory Protocol and Worksheet

**Table 1. Glucose Results Before And After Adding Lactex®**

Sample Number	Color of test strip <u>BEFORE</u> adding lactase, <u>AFTER</u> dipping in milk samples	Relative amount of glucose (mg/dl) <u>BEFORE</u> adding lactase	Color of test strip <u>AFTER</u> adding lactase, <u>AFTER</u> dipping in milk samples	Relative amount of glucose (mg/dl) <u>AFTER</u> adding lactase	Milk Type
1					
2					
3					

### Analysis Questions – Part 1

1. What is the purpose of the glucose test strips?

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2. What effect does Lactex® have on cow milk?

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3. Which sample is the cow milk? How do you know?

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4. Can Lactex® be used specifically on lactose? Are there any residual effects on the other sugars?

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## BIOTECHNOLOGY Laboratory

pH=0

pH=7

pH=14

Acidic

Neutral


Basic

## Part 2: Determine Effect of pH on Lactase Activity

Independent Variable: \_\_\_\_\_

Dependent Variable: \_\_\_\_\_

### Add lactase to the appropriate tubes


1. Each station has 5 tubes containing 1 ml of pH solutions "2", "4", "7", "10", and "12" and one tube containing "dH<sub>2</sub>O." All should be labeled appropriately and legibly.
2. Identify the tube labeled "Lactex®" and mix gently by inversion to get an even distribution of the powder through the solution.
3. Do not shake vigorously or produce bubbles as this may damage the enzyme.
4. Using the pipette labeled "Lactex®," add 1 ml of the solution to all 6 tubes.
5. **DO NOT TOUCH** the end of the pipette inside the tubes or your samples will be contaminated. Use proper pipette technique.
6. Vortex each sample for **ONLY 2 seconds**. Be sure the vortex speed is not set over 9.
7. Incubate all samples for 3-5 minutes at room temperature. 

We are now certain the Lactex® tablet breaks down only lactose. They have asked you as the scientist to determine under what conditions it will work. Since the tablet is ingested, it must be determined whether or not the Lactex® tablet can remain effective through a wide range of pH values that it will encounter in the human digestion tract. Using the cow milk identified in Part I, your team will conduct an experiment to test the activity of the Lactex® tablet across a range of pH values.


## LOOKING INTO LACTASE

### BIOTECHNOLOGY Laboratory Protocol and Worksheet

#### **Add cow milk to tubes:**

8. Using the pipette labeled “cow milk,” add 2 ml of cow milk to all 6 tubes.
9. **DO NOT TOUCH** the end of the pipette inside the tubes or your samples will be contaminated (use proper pipette technique).
10. Vortex the samples 1-6 for **ONLY 2 seconds**.
11. Incubate all samples for 3-5 minutes at room temperature. 

#### **Test for glucose:**

12. Label six glucose test strips “2”, “4”, “7”, “10”, and “12” and “dH<sub>2</sub>O”.
13. Take glucose test strip “2” and dip it into new tube “2” (get the test pad wet).
14. Immediately remove the test strip and lay it down on the paper towel. Repeat for the remaining samples. Keep the strips separated on the paper towels.
15. Wait 3 minutes. 
16. Record color of the test strip and corresponding percentage of the amount glucose in **Table 2**.

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pH	Color of strip	Relative amount of glucose (0-3000mg/dl) after adding lactase
2		
4		
7		
10		
12		
dH <sub>2</sub> O control		

### Analysis Questions – Part 2

1. Why do we use the water sample?

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2. What is the optimum pH range for Lactex®? How do you know?

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3. What effect does changing the environment from an alkaline (basic) environment to an acidic environment have on the activity of the lactase enzyme?

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4. Why did you add lactase enzyme to the 6 test tubes and incubate it BEFORE adding the milk?

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## BIOTECHNOLOGY Laboratory Protocol and Worksheet

### Part 3: Determine Effect of Temperature on Lactase Activity

Independent Variable: \_\_\_\_\_

Dependent Variable: \_\_\_\_\_

#### Set up the tubes for temperature incubation

1. Each station has six test tubes labeled: **HL** (hot Lactex®), **HM** (hot milk), **RTL** (room temp Lactex® ), **RTM** (room temp milk), **CL** (cold Lactex®), and **CM** (cold milk).

#### Put your group number on all tubes.

2. Identify the tube labeled Lactex® and again **mix gently** by inversion to get an even distribution of the powder through the solution.
3. Using the pipette labeled **Lactex®**, add **1 ml** of lactase solution to the three tubes labeled: **HL**, **RTL**, and **CL**
4. Using the pipette labeled **cow milk**, add **2 ml** of cow milk to tubes labeled: **HM**, **RTM**, **CM**. Place the test tubes at their respective temperatures for 10 minutes: **(NO CAPS)**
  - a. hot tubes **HL** and **HM** into the hot water
  - b. cold tubes **CL** and **CM** into the ice
  - c. room temperature tubes **RTL** and **RTM** leave in the rack at your station.
5. Record the hot water temperature, the ice temperature, and room temperature in °C in **Table 3**.
6. Carefully pour the contents of the **HM** tube into the **HL** tube.
7. **DO NOT SPILL**. Tubes and contents will be hot so use appropriate laboratory safety techniques, hold tubes at the top.

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8. Carefully pour the contents of the **CM** tube into the **CL** tube. **DO NOT SPILL.**
9. Carefully pour the contents of the **RTM** tube into the **RTL** tube. **DO NOT SPILL.**
10. Cap all the tubes and invert to mix a few times but do not shake vigorously.
11. Place each tube back into its appropriate temperature for 5 minutes (**NO CAPS**).
12. Label 3 new glucose strips **H** for Hot, **C** for Cold, and **RT** for Room Temperature.
13. Take glucose test strip **H** and dip it into tube **HL** (just enough to get the test pad wet). Immediately remove the test strip and lay it down on the paper towel.
14. Take glucose test strip **C** and dip it into tube **CL** (just enough to get the test pad wet). Immediately remove the test strip and lay it down on the paper towel.
15. Take glucose test strip **RT** and dip it into tube **RTL** (just enough to get the test pad wet). Immediately remove the test strip and lay it down on the paper towel.
16. **Wait 3 minutes** and record color of the test strip and corresponding glucose amount in

**Table 3.**



17. After completing the experiment, as a post-laboratory activity you will generate a line graph that shows the dependence of enzyme activity on temperature. Be sure to put a title on the graph and label the axes.

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## BIOTECHNOLOGY Laboratory Protocol and Worksheet

**Table 3: Temperature and Glucose Results**

Temperature Condition	Actual Temperature (°C)	Color of strip	Relative amount of glucose (0-3000mg/dl) after adding lactase
Ice			
Room temperature			
Hot water			

### Analysis Questions– Part 3

1. Which temperature represents the control? Why?

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2. What is the optimum temperature for Lactex®? How do you know?

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3. Explain the difference in enzyme activity at varying temperatures. What happened to the enzyme?

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4. Do you think it possible to recover the enzyme activity from the ones treated to extreme temperature conditions? Why or why not?

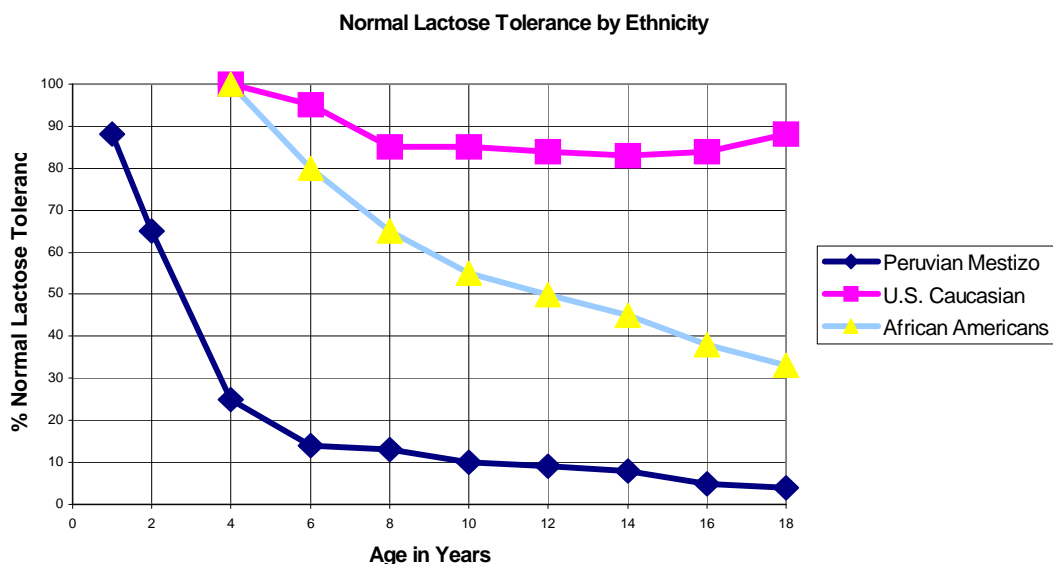
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# LOOKING INTO LACTASE

## BIOTECHNOLOGY Laboratory Protocol and Worksheet

- Using the graph, write a brief paragraph comparing the similarities and differences in the three populations in terms of normal lactose tolerance.



- Using the data in the chart, construct a graph. Be sure to follow customary rules for graphing, including an appropriate title and labeled axes. Put the independent variable on the x axis and the dependent variable on the y axis. Then write a paragraph comparing the similarities and differences in the three populations in terms of normal lactose tolerance.

Normal Lactose Tolerance in U.S. Caucasian and African American Children and Peruvian Mestizo Children (in %)

Age in Years	Caucasian	African American	Peruvian
2	No data	No data	65
4	100	100	25
6	95	80	14
8	85	65	13
10	85	55	10
12	84	50	9
14	83	45	8
16	84	38	5
18	88	33	4

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## BIOTECHNOLOGY Laboratory Protocol and Worksheet

3. Propose a solution to the problem of school-age children in Baltimore City needing calcium and not making use of the milk provided with school lunches (due to their symptoms of lactose intolerance). Write this up in the form of a letter to the principal.
  
4. While on a field trip with your biology class to visit a local farm, you accidentally become separated from your classmates and miss lunch. After a while, you get so hungry that you pull up some grass and eat it. Shortly afterward, you were reunited with your classmates. On the bus ride home a few hours later, you developed terrible cramps and gas and nobody wanted to sit near you. You don't understand what happened. You see the cows, sheep, and goats eating grass, and they are mammals just like you. You asked your teacher about it, and she said that many herbivorous animals have compartmentalized stomachs that contain certain bacteria and protozoa. She also mentioned that humans don't have the ability to digest cellulose, a carbohydrate found in the cell walls of plants.
  - a. In terms of enzymes, explain why eating the grass caused you to have digestive problems.

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- b. Explain the role of bacteria and the protozoa in the stomachs of herbivorous mammals such as cows, sheep, and goats. What do you think would happen to these animals if they did not have this symbiotic relationship with the bacteria and the protozoa?

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# LOOKING INTO LACTASE

## BIOTECHNOLOGY Laboratory Protocol and Worksheet

### Multiple Choice Questions

1. The enzyme \_\_\_\_\_ can break down \_\_\_\_\_ into \_\_\_\_\_ and galactose.
  - a. lactose, glucose, lactase
  - b. lactase, lactose, glucose
  - c. glucase, lactase, lactose
  - d. lactase, glucose, lactose
2. Enzymes are \_\_\_\_\_ molecules which \_\_\_\_\_ specific chemical reactions.
  - a. carbohydrate, inhibit
  - b. lipid, speed-up
  - c. protein, speed-up
  - d. nucleic acid, inhibit
3. The ability of an enzyme to function is influenced by the
  - a. indicator
  - b. temperature
  - c. enzyme shape
  - d. both b and c
4. The presence of glucose can be shown by
  - a. a glucose test strip turning from yellow to green
  - b. a lactose test strip turning from green to yellow
  - c. a sample of milk turning from blue to yellow
  - d. a sample of milk turning from blue to red
5. Which of the following groups of people retain into adulthood, with the largest percent, the natural ability to digest lactose
  - a. Asians
  - b. Africans
  - c. Latinos
  - d. Northern Europeans

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## BIOTECHNOLOGY Laboratory Protocol and Worksheet

### Short Answers

6. Which is the best temperature for breaking down lactose in the milk, using Lactex® tablets — freezing, room temperature, or boiling?

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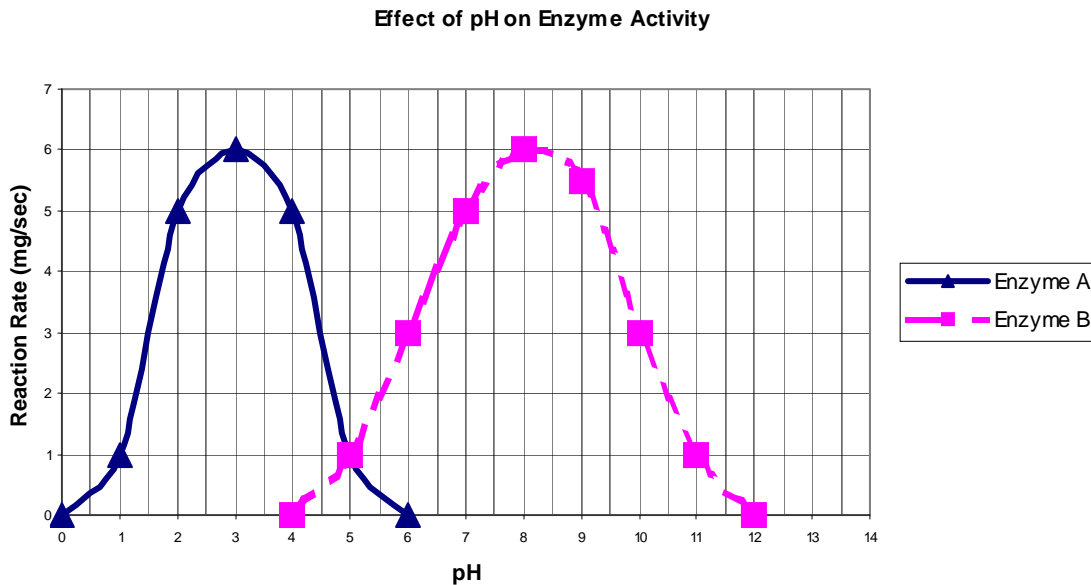
7. What does this suggest about how effective the Lactex® tablets would be for someone who likes ice cream compared with someone who drinks milk?

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8. Look at the following graph showing the reaction rates of two enzymes.

#### Effect of pH on Enzyme Activity



a. Which enzyme works better in an alkaline environment? \_\_\_\_\_

b. At which pH do the two enzymes work equally well? \_\_\_\_\_

c. What is the optimum pH for Enzyme A? \_\_\_\_\_

d. Describe the overall reaction rate pattern shown by both enzymes.

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