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Datum Sheet for Laboratory 5
Measurement of β-Galactosidase Activity in Lactaid™ Tablets

A. Extraction of β-Galactosidase (Lactase) Activity from Lactaid™ Tablets

1. Which form of Lactaid did you use for this experiment? (0.5 points)

2. What did the label indicate about the activity of the enzyme per tablet or caplet? (0.5 points)

B. Construction of an o-Nitrophenol Standard Curve

1. What is the purpose of making a standard curve? (0.5 points)

2. Enter your raw data for the construction of the standard curve (2 points).

Table 5.3. Datum Collection for o-Nitrophenol Standard Curve

Tube	A(420 nm)	1.0 mM o-nitrophenol (mL)	o-nitrophenol (nmoles)
1		0	
2		0.1	
3		0.2	
4		0.3	
5		0.4	
6		0.5	
7		0.6	
8		0.7	
9		0.8	
10		0.9	
11		1.0	

3. **Attach to this datum sheet a graph of your standard curve.** You can make a graph with a software such as Excel if you like. (2 points)

4. Give the conversion factor (slope of the line) that you determined from this standard curve (0.5 points).

1 nmole = _____A

C. Preliminary Measurement of β-Galactosidase (Lactase) Activity in Lactaid™ Tablets

1. Show your raw data for the measurement of β-galactosidase activity in each of the dilutions of the Lactaid™ extract (3 points).

Table 5.4. Datum Collection: Preliminary Lactaid™ extract activity measurement

Tube	Lactase Solution	Time Reaction Started	Time Reaction Stopped	Total Time	A(420 nm)	nmoles o-nitrophenol
1	None					
2	Undiluted					
3	10^{-1} Dilution					
4	10^{-2} Dilution					
5	10^{-3} Dilution					
6	10^{-4} Dilution					

2. For each reaction in which you could accurately measure the absorbance and the time, show your calculations of the activity in **nmoles/min ml** (3 points)

3. Fill in the following table to summarize these data (0.5 point each, 2.5 points total).

Table 5.5. Summary of Preliminary Lactaid™ Activity Measurements

Lactase Solution	Activity (nmoles/min mL)
Undiluted	
10^{-1} Dilution	
10^{-2} Dilution	
10^{-3} Dilution	
10^{-4} Dilution	

4. Does the activity change with dilution as expected? Why might this not have occurred? (0.5 point)

5. Which dilution did you decide to use for the rest of the experiments? (1 point)

   _____

D. Precise Measurement of β-Galactosidase (Lactase) Activity in Lactaid™ Tablets

1. Give the raw data for the three replicate assays of the selected dilution of lactase (4 points).

   Dilution used: _____
   Volume used: _____ ml

Datum Collection: Precise Measurement of Lactaid™ Extract Activity (From Table 5.5 in lab manual)

Tube	Time Started	Time Stopped	Total Time Elapsed	A (420nm)	nmoles
2					
3					
4					

2. Show your calculations for the activity in the original extract in **nmoles/min ml** for each of the three reactions. (2 points)

3. Give the mean activity and the standard deviation for the original extract in **nmoles/min ml** and in **µmoles/min ml** (2 points).

   _____ nmoles/min mL

   _____ µmoles/min mL

4. Show your calculations for the total amount of β-galactosidase activity in a Lactaid™ tablet (1 point).

5. How did your Lactaid sample compare to the other forms of Lactaid which were available for the class to use? (1 point)

E. Additional Questions (1 point each, 4 points total)

1. Into which of the six major enzyme groups should the enzyme β-galactosidase be placed?

2. Suppose you make an extract of a Lactaid tablet and obtain 5.5 ml of solution. When you assay this extract for β-galactosidase activity, you find that 47 nmoles are made using 0.05 ml of a 10^{-3} dilution in 3.48 minutes using the standard protocol described in this experiment. **What is the activity of the tablet?**

3. While we have assumed that product formation in the β-galactosidase assay is linear with time, this is not always a safe assumption. Suppose you are measuring the activity of a different enzyme called L-lactate dehydrogenase and measure the absorbance of the reaction at 15 second intervals for two minutes. You obtain the following results:

Time (seconds)	Absorbance
0	0
15	0.05
30	0.10
45	0.15
60	0.20
75	0.24
90	0.27
105	0.30
120	0.32

Make of a graph of these data using Excel. **Attach a copy of your graph to this datum sheet.** Over what time interval is product formation linear with time? What is the rate of product formation as a ΔA/min in this time interval?

4. The actual molar extinction coefficient for o-nitrophenol has been calculated and used to develop a simple formula for calculating β-galactosidase activity. This formula is

$$\text{Activity Units} = \frac{A_{420} \times 0.380}{\text{minutes at 37 C}}$$

where one unit of activity is the amount of enzyme needed to convert 1 μmole of ONPG to o-NP in one minute.

Suppose a preparation of β-galactosidase was made in the laboratory. One μl of this preparation was added to 99 μl of buffer. 10 μl of this solution was then added to 1.0 ml of reaction buffer and equilibrated to 37 C. 0.2 ml of ONPG was added and after 11 minutes, the reaction was stopped by added 0.5 ml of sodium carbonate. The absorbance of the solution was then found to be 0.412. **What was the enzyme activity in the original solution in units/ml?**