BCH 372 Names: _____
Modern Concepts in Biochemistry Laboratory _____
(30 points) _____
_____

Datum Sheet for Laboratory 4
Assay for L-Lactate Dehydrogenase

1. Attach to this datum sheet the graphs of the rate plots for the three **replicate** samples of the stock or diluted LDH solution, using 0.15 M CAPS, pH 10.0 as the buffer and 150 mM L-lactate as the substrate. You do not need to include any preliminary trials. You can make the graphs with graph paper or with Excel (4 points).

   Volume and dilution of stock enzyme used: _____

   Volume of water used: _____

2. Summarize the results in the following chart (3 points):

assay	V_o (ΔA_{340}/second)	V_o (ΔA_{340}/min)
1	_____	_____
2	_____	_____
3	_____	_____

3. Show your calculation of the average initial velocity (V_o) in A_{340}/min (1 point).

4. Show your calculation of the average initial velocity (V_o) in μmoles/min (1 point).

5. Show your calculation of the activity of the stock LDH solution in units/ml enzyme (2 points).

6. Summarize your results for the reactions in which L-lactate was replaced with D-lactate and NAD^+ was replaced with $NADP^+$. Was there activity with the alternative substrate? If there was activity, compare the activity in nmoles/min ml enzyme to that with the standard substrate. Show all of the relevant data and calculations (4 points).

7. Attach to this datum sheet the graphs of the rate plots for the three replicate samples of the diluted LDH solution with each of your two buffer solutions at different pHs (4 points).

8. Summarize the results in the following chart (4 points).

Buffer 1: _____

assay	volume of enzyme	volume of water	initial velocity ΔA_{340}/min	average initial velocity
1				
2				
3				

Buffer 2: _____

assay	volume of enzyme	volume of water	initial velocity ΔA_{340}/min	average initial velocity
1				
2				
3				

9. Show your calculations of activity in units/ml enzyme for each of your two buffers (2 points).

Buffer 1: _____

Buffer 2: _____

10. Summarize the class data for the effects of buffers on the rate of the LDH reaction (2 points).

buffer	pH	LDH activity (units/ml)

11. Attach to this datum sheet a pH optimum curve in which you plot the units/ml of the stock LDH solution as a function of pH (2 point)

12. What conclusions can you draw from these data? (1 point)