

BCH 372  
Modern Concepts in Biochemistry Laboratory  
(30 points)

Names: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

## Datum Sheet for Laboratory 3 Spectrophotometry

1. Attach to this datum sheet a series of graphs showing the absorption spectra of 1 mM solutions of Na pyruvate, Na L-Lactate,  $\text{NAD}^+$ , and NADH. Use the actual data generated by the spectrophotometer (2 points).
2. What are the wavelengths of maximal absorbance for each compound? (0.5 points each, 2 points total)

Na pyruvate \_\_\_\_\_

Na L-lactate \_\_\_\_\_

$\text{NAD}^+$  \_\_\_\_\_

NADH \_\_\_\_\_

3. Show your analysis of the stock 1.0 mM solution of NADH solution (1 point each, 5 points total).
  - a. initial absorbance at 340 nm: \_\_\_\_\_
  - b. absorbance after dilution (give dilutions used)

dilution	_____	absorbance	_____
dilution	_____	absorbance	_____
dilution	_____	absorbance	_____
  - c. Give your calculation of the NADH concentration in the 1.0 mM solution:

- d. how close is this value to 1.0 mM?
- e. how can you explain any differences between the observed value and expected value?

4. Give the raw data for the preparation of the NADH standard curve using the 1.0 mM stock solution (4 points).

<u>tube</u>	<u>0.15 M CAPS</u>	<u>1.0 mM NADH</u>	<u>A(340 nm)</u>	<u>ave</u>	<u>μmoles NADH</u>
1	1000 μl	0	_____	_____	_____
2	990	10 μl	_____	_____	_____
3	990	10 μl	_____	_____	_____
4	980	20 μl	_____	_____	_____
5	980	20 μl	_____	_____	_____
6	950	50 μl	_____	_____	_____
7	950	50 μl	_____	_____	_____
8	900	100 μl	_____	_____	_____
9	900	100 μl	_____	_____	_____
10	850	150 μl	_____	_____	_____
11	850	150 μl	_____	_____	_____
12	800	200 μl	_____	_____	_____
13	800	200 μl	_____	_____	_____

14	700	300 $\mu$ l	_____	_____	_____
15	700	300 $\mu$ l	_____		
16	600	400 $\mu$ l	_____	_____	_____
17	600	400 $\mu$ l	_____		
18	500	500 $\mu$ l	_____	_____	_____
19	500	500 $\mu$ l	_____		
20	400	600 $\mu$ l	_____	_____	_____
21	400	600 $\mu$ l	_____		

5. Attach to this datum sheet a graph of the NADH standard curve. You can make the graph either on a piece of graph as provided in the lab or with a software package such as Excel (2 points).

Give the conversion factor relating  $A_{340}$  and  $\mu$ moles of NADH (1 point).

\_\_\_\_\_  $A_{340}/\mu$ mole

6. Give the raw data for the analysis of the unknown NADH solutions (2 points)

Solution                      concentrated   1/4      1/16      other dilutions (indicate dilution factor)

Unknown \_\_\_\_\_      \_\_\_\_\_      \_\_\_\_\_      \_\_\_\_\_      \_\_\_\_\_

Unknown \_\_\_\_\_      \_\_\_\_\_      \_\_\_\_\_      \_\_\_\_\_      \_\_\_\_\_

7. Show your calculations of the NADH concentration of the two unknown solutions. Give the results obtained both with the NADH standard curve and with the Beer-Lambert Law (2 points each, 4 points total).

Unknown \_\_\_\_\_

Unknown \_\_\_\_\_

8. Answer the following questions from pages 80-81 of the lab manual. SHOW YOUR CALCULATIONS AND PUT THE ANSWER IN A BOX. (1 point each, 3 points total)

1.

2.

3.

9. Answer the following questions from pages 87 of the lab manual. SHOW YOUR CALCULATIONS AND PUT THE ANSWER IN A BOX. (1 point each, 5 points total)

1.

2.

3.

4.

5.