

BCH 372  
 Modern Concepts in Biochemistry Laboratory  
 (30 points)

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

## Datum Sheet for Laboratory 13 Affinity Chromatography

1. Give the LDH activity of the dialyzed resuspended 65% ammonium sulfate pellet fraction (**D65P**). Show the volume assayed and any dilution, the initial velocities of the three replicate runs, the mean, and the activity (5 points).

volume assayed (dilution) \_\_\_\_\_

$V_o$  values ( $\Delta A_{340}/\text{min}$ ) \_\_\_\_\_

average  $V_o$  ( $\Delta A_{340}/\text{min}$ ) \_\_\_\_\_

average  $V_o$  ( $\mu\text{moles}/\text{min}$ ) \_\_\_\_\_

activity ( $\mu\text{moles}/\text{min ml}$ ) \_\_\_\_\_

2. Give the LDH activities in  $\Delta A_{340}/\text{min}$  and  $\mu\text{moles}/\text{min}$  for the 50  $\mu\text{l}$  volumes of the fractions from the Cibacron Blue agarose affinity column. Use as many lines as necessary (6 points).

<u>fraction #</u>	<u>rate (<math>\Delta A_{340}/\text{min}</math>)</u>	<u>rate (<math>\mu\text{moles}/\text{min}</math>)</u>	<u>activity (<math>\mu\text{moles}/\text{min ml}</math>)</u>
1	_____	_____	_____
2	_____	_____	_____
3	_____	_____	_____
4	_____	_____	_____
5	_____	_____	_____
6	_____	_____	_____
7	_____	_____	_____
8	_____	_____	_____

9	_____	_____	_____
10	_____	_____	_____
11	_____	_____	_____
12	_____	_____	_____
13	_____	_____	_____
14	_____	_____	_____

- Attach to this datum sheet an elution profile for the Cibacron Blue agarose column, in which you plot the LDH activity in  $\mu\text{moles/min ml}$  as a function of fraction number (2 points).
- Give the following information about the pooled fraction (AC pool) from this column (7 points).

fractions pooled \_\_\_\_\_

volume of pool (ml) \_\_\_\_\_

volume assayed \_\_\_\_\_

$V_o$  values ( $\Delta A_{340}/\text{min}$ ) \_\_\_\_\_

average  $V_o$  ( $\Delta A_{340}/\text{min}$ ) \_\_\_\_\_

average  $V_o$  ( $\mu\text{mole}/\text{min}$ ) \_\_\_\_\_

activity ( $\mu\text{moles}/\text{min ml}$ ) \_\_\_\_\_

- Attach to this datum sheet the graphs used to calculate the  $V_o$  for this fraction (1 point).
- Calculate the percent recovery in the pooled fraction based on loading **0.5 ml** of the **D65P fraction** (1 point).

7. Answer the following questions from the Additional Problem Set on page 177 (1 point each for questions # 1-6, 2 points for # 7; 8 points total).

1.

2.

3.

4.

5.

6.

7.