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

Datum Sheet for Laboratory 7 and Laboratory 8
Ammonium Sulfate Fractionation of
L-Lactate Dehydrogenase, Part A and Part B

1. Provide the following information about the purification of LDH from your crude extract by ammonium sulfate fractionation (0.5 point each, 4 points total).

   organ source of crude extract _____

   volume (ml) of crude extract (**CE**) _____

   g of Ammonium Sulfate added during first precipitation _____

   volume (ml) of 40% AS supernatant (**fraction 40S**) _____

   volume (ml) of 40% AS pellet fraction (**fraction 40P**) _____

   g of Ammonium Sulfate added during second precipitation _____

   volume (ml) of 65% AS supernatant (**fraction 65S**) _____

   volume (ml) of 65% AS pellet fraction (**fraction 65P**) _____

2. Provide all of the key information on your LDH enzyme assays of these fractions. Include the data on the crude extract from **Laboratory 6** (1 point each, 5 points total).

fraction	volume assayed	dilution factor (if any)	individual velocity values (A_{340}/min)	mean initial velocity (A_{340}/min)
crude extract	_____	_____	_____	_____
40% AS supernatant	_____	_____	_____	_____
40% AS pellet	_____	_____	_____	_____
65% AS pellet	_____	_____	_____	_____
65% AS supernatant	_____	_____	_____	_____

3. For each fraction that you assayed, calculate the **rate** of the reaction in **µmoles/min**. Then calculate the LDH **activity** of each fraction in **µmoles/min ml** (**units/ml)**. See the sample calculations in Laboratory 4 and on pages 94-96 and 99 of the lab manual. Show your calculations below (0.5 point each, 2.5 points total).

crude extract

40% AS supernatant fraction:

40% AS pellet fraction:

65% AS pellet fraction:

65% AS supernatant fraction:

4. Now calculate the **total amount of activity** in each fraction in **enzyme units (µmoles/min)**. **Multiply the activity of each fraction by its volume**. Show your calculations below (0.5 points each, 2.5 points total).

crude extract:

40% AS supernatant:

40% AS pellet fraction:

65% AS pellet fraction:

65% AS supernatant fraction:

5. Finally, determine the **percent recovery** in each fraction: **divide the total units in each fraction by the total units in the crude extract and multiply by 100%** (0.5 points each, 2.5 points total).

crude extract

40% AS supernatant:

40% AS pellet fraction

65% AS pellet fraction:

65% AS supernatant fraction:

6. Give the raw data for the preparation of the BSA standard curve (2 points).

volume BSA (µl)	amount of BSA (µg)	absorbance values		average
0	_____	_____	_____	_____
5	_____	_____	_____	_____
10	_____	_____	_____	_____
15	_____	_____	_____	_____
20	_____	_____	_____	_____
30	_____	_____	_____	_____
40	_____	_____	_____	_____
50	_____	_____	_____	_____
60	_____	_____	_____	_____

7. Attach to this datum sheet a graph of the BSA 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 (1 point).

8. Define a conversion factor relating absorbance to the amount of protein (0.5 point).

   1 μg = _____A_{595}

9. Give the raw data for the analysis of the unknown protein solutions from the ammonium sulfate fractionation. Using your standard curve and the conversion factor, calculate the amount of protein in μg in each tube. **Note that you can only use the absorbance values that fall within the linear range of the standard curve to calculate a protein amount or concentration** (2 points).

tube	water	sample	volume	A_{595}	μg protein
1	100 μl	none	0 μl	_____	_____
2	90	fraction CE	10	_____	_____
3	70	fraction CE	30	_____	_____
4	90	fraction CE 1/10	10	_____	_____
5	70	fraction CE 1/10	30	_____	_____
6	90	fraction CE 1/100	10	_____	_____
7	70	fraction CE 1/100	30	_____	_____
8	90	fraction 40P	10	_____	_____
9	70	fraction 40P	30	_____	_____
10	90	fraction 40P 1/10	10	_____	_____
11	70	fraction 40P 1/10	30	_____	_____
12	90	fraction 40P 1/100	10	_____	_____
13	70	fraction 40P 1/100	30	_____	_____
14	90	fraction 40S	10	_____	_____
15	70	fraction 40S	30	_____	_____
16	90	fraction 40S 1/10	10	_____	_____
17	70	fraction 40S 1/10	30	_____	_____
18	90	fraction 40S 1/100	10	_____	_____
19	70	fraction 40S 1/100	30	_____	_____
20	90	fraction 65P	10	_____	_____
21	70	fraction 65P	30	_____	_____
22	90	fraction 65P 1/10	10	_____	_____
23	70	fraction 65P 1/10	30	_____	_____
24	90	fraction 65P 1/100	10	_____	_____
25	70	fraction 65P 1/100	30	_____	_____

26	90	fraction 65S	10	_____	_____
27	70	fraction 65S	30	_____	_____
28	90	fraction 65S 1/10	10	_____	_____
29	70	fraction 65S 1/10	30	_____	_____
30	90	fraction 65S 1/100	10	_____	_____
31	70	fraction 65S 1/100	30	_____	_____

10. Show your calculations of the **average protein concentration** of each fraction (0.5 point each, 2.5 points total).

fraction CE:

fraction 40P

fraction 40S

fraction 65P

fraction 65S

11. Now calculate the **specific activity** of each fraction in units/mg by **dividing the activity in µmoles/min ml by the protein concentration in mg/ml** (0.5 point each, 2.5 points total).

fraction CE:

fraction 40P

fraction 40S

fraction 65P

fraction 65S

12. Finally, calculate the **purification factor** for each fraction by **dividing the specific activity of that fraction by the specific activity in the crude extract. P**ut all of the data together in the following summary table (3 points).

Summary of Ammonium Sulfate Fraction of LDH Activity in Crude Extract

fraction	volume (ml)	LDH units/ml	total units	% recovery	protein mg/ml	LDH sp. act.	fold purification
crude extract							
40% AS pellet							
40% AS supernatant							
65% AS pellet							
65% AS supernatant							