Names: _____
_____
_____
_____

Datum Sheet for Labs 11 - 13
Analysis of Proteins in Different Animal Organs,

A. Labs 11 through 13 focus on analyzing proteins in the following tissues:

PK:	porcine kidney	CL:	calf liver
CB:	chicken breast muscle	PH:	porcine heart
BP:	bovine pancreas	PB:	porcine brain

1. Which tissue would you expect to have the highest concentration of protein? Why? (1 point)

2. Which tissue would you expect to have the highest level of L-lactate Dehydrogenase Activity? Why? (1 point)

PART A

B. Preparation of Acetone Powder Suspensions (1.5 points)

1. Which organ acetone powder did you use? _____

2. How many mg of powder did you use? _____

3. About how many ml of supernatant did you obtain? _____

C. Protein Concentrations of Acetone Powder Suspensions

1. Fill in the following chart with the data for the BSA standard curve. Please note that the number of tubes used will depend on how you set up your standard curve. (1.5 points)

tube	volume of water	volume of BSA	µg of BSA	A_{595}	average ABS_{595}
1	_____	_____	_____	_____	_____
2	_____	_____	_____	_____	_____
3	_____	_____	_____	_____	
4	_____	_____	_____	_____	_____
5	_____	_____	_____	_____	
6	_____	_____	_____	_____	_____
7	_____	_____	_____	_____	
8	_____	_____	_____	_____	_____
9	_____	_____	_____	_____	
10	_____	_____	_____	_____	_____
11	_____	_____	_____	_____	
12	_____	_____	_____	_____	_____
13	_____	_____	_____	_____	
14	_____	_____	_____	_____	_____
15	_____	_____	_____	_____	

2. Attach to this datum sheet your graph of the BSA standard curve. (1 point)

3. Using your standard curve, determine the conversion factor for relating absorbance at 595 nm to µg of protein and provide it in the space below. (1 point)

_____

4. Fill in the following chart with the data for the protein assay of the acetone powder suspension. Calculate µg amount using conversion factor in #3 for those tubes that are in the range of your standard curve. (1 point)

Tube	Solution	Water (µL)	Extract (µL)	Coomassie Plus Reagent (mL)	A_{595}	µg
1	---	50	0	3		
2	Stock Solution	45	5	3		
3		40	10	3		
4		30	20	3		
5		0	50	3		
6	1/10 Dilution	45	5	3		
7		40	10	3		
8		30	20	3		
9		0	50	3		
10	1/100 Dilution	45	5	3		
11		40	10	3		
12		30	20	3		
13		0	50	3		

5. Choose three representative tubes from above and calculate the concentration of protein extract in mg/ml based on that tube. Indicate the tubes you have chosen and show your calculations (1.5 points)

Tube #	µg	Calculations	mg/ml
_____	_____		_____
_____	_____		_____
_____	_____		_____

6. Using the three representative tubes from #5, give the average value of the protein concentration of your acetone powder suspension in mg/ml and show your calculations. (1 point)

7. Using the three representative tubes from #5, give the standard error of the protein concentration of your acetone powder suspension in mg/ml and show your calculations. (1 point)

Lab Exercise 12: Protein Analysis PART B

A. Preparation of Protein Samples

1. Give the name of the tissue extract and the protein concentration that you determined last week. (1 point)

Tissue Extract: _____

Protein Concentration (mg/ml): _____

2. Give the calculated dilution factor you used to make a 2 mg/ml solution. (1 point)

_____

3. Give the amount of extract and the amount of water you used to prepare 300 µl of a working solution at 2 mg/ml. (1 point)

extract: _____

water: _____

C. Analysis of Standard Protein Gels

1. Attach to this datum sheet the printed digital image of the SDS-polyacrylamide gel. Be sure that you label the lanes number the bands in your gel images. (2 points)

2. Fill in the following chart to indicate the mobilities in (cm) of each band in the Precision Plus Protein Standard and the purified proteins on the gel that was made by your group. Measure the distance in **cm** from the bottom of each well to the center of each band. Use **NV** to indicate that a particular protein band was not visible. (2 points)

Precision Plus Protein Standard**:**

Band Color	molecular mass	mobility (cm) gel A	gel B
Blue	10,000	_____	_____
Blue	15,000	_____	_____
Blue	20,000	_____	_____
Pink	25,000	_____	_____
Blue	37,000	_____	_____
Blue	50,000	_____	_____
Pink	75,000	_____	_____
Blue	100,000	_____	_____
Blue	150,000	_____	_____
Blue	250,000	_____	_____

3. Attach to this datum sheet the standard curve for the proteins in the Protein standard for each of the 4-15% polyacrylamide gels. **Plot mobility (in cm) on the X axis and log molecular weight on the Y axis. Draw the best fit line you can through the data. (2 points) You should have a separate standard curve for each gel.**

4. **Practice Question:** You are provided a protein that has a quaternary structure composed of three subunits. The molecule mass of each of the three subunits is as follows:

   Subunit A 17,000 daltons
   Subunit B 25,000 daltons
   Subunit C 30,000 daltons

   If this protein was fractionated on a SDS-PAGE gel like you did in class, what would you expect in the lane that contained this protein? Be specific, include number and size of bands and also the expected distance migrated based on your standard curve from #3. (2 points)

Lab Exercise 13: Protein Analysis PART C

1. Attach to this datum sheet the graphs for each of the three LDH Assay replicates. (2 points)

2. Using your graphs, calculate the change in absorbance (ΔA_{340}) per minute. Be sure to correct for the change in absorbance for the control reaction (1.5 points)

   Replicate 1 _____ ΔA_{340}/minute

   Replicate 2 _____ ΔA_{340}/minute

   Replicate 3 _____ ΔA_{340}/minute

3. Average the three values from above and then calculate the amount of activity in ΔA_{340}/min ml. Use the conversion factor of 0.00622 A_{340}/nmole to express the activity of the tissue homogenate or acetone powder suspension in nmoles/min ml. Be sure to multiply by 3 since the total reaction volume was 3.0 ml. **(2 points)**

4. Calculate the specific activity of L-lactate dehydrogenase for your organ acetone powder suspension. Specific activity will have units of nmoles/min mg protein. **(2 points)**