

BCH 372
 Modern Concepts in Biochemistry Laboratory
 (30 points)

Names: _____

Datum Sheet for Laboratories 9 and 10

Western Blot Analysis of L-Lactate Dehydrogenase

1. Indicate the protein concentrations, dilution factors, and the volumes used to prepare 100 μl of each fraction at a concentration of 2 mg/ml to use as a sample for the nondenaturing gel electrophoresis (2 points).

<u>fraction</u>	<u>protein conc. (mg/ml)</u>	<u>dilution factor to give 2 mg/ml</u>	<u>vol (μl) of fraction used</u>	<u>vol (μl) of water</u>
CE	_____	_____	_____	_____
40P	_____	_____	_____	_____
40S	_____	_____	_____	_____
65P	_____	_____	_____	_____
65S	_____	_____	_____	_____

2. Give the conditions used for the electrophoresis of the samples under nondenaturing conditions (0.5 point each, 1 point total).

Voltage _____

Approximate Total Time _____

3. Attach to this datum sheet a copy of the image of your Coomassie Blue stained polyacrylamide gel. Label the image to identify the sample added to each lane (2 points).
4. Attach to this datum sheet the two standard curves prepared from the molecular weight markers (1 point each, 2 points total).

65P

65S

H-LDH

M-LDH

7. What is the native molecular mass of LDH? Did you see band with this mass in your samples? Was it purified? (2 points)

8. Attach to this datum sheet a copy of the image of the polyacrylamide gel used in the Western Blot. Did it indicate that proteins were transferred to the PVDF membrane? (2 point)
9. Attach to this datum sheet a copy of the image of your Western Blot. Label the image to identify the sample added to each lane (2 points).
10. Describe what you see in the Western Blot. Did it work? Can you see visible bands in the standard LDH preparations? Can you see visible bands in the samples from your LDH purification? Do these bands match any particular bands in the Coomassie Blue stained gel? (4 points)
11. Answer the following questions from the Additional Problem Set on page 293 of the lab manual (1 point each, 6 points).
 - 1.
 - 2.
 - 3.
 - 4.
 - 5.
 - 6.