

BCH 372
 Modern Concepts in Biochemistry Laboratory
 (30 points)

Names: _____

Datum Sheet for Laboratory 14 Completion of LDH Purification

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

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

2. 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).
3. Define a conversion factor relating absorbance to the amount of protein (1 point).
- 1 μ g = _____ A_{595}
4. Make a graph comparing this standard curve with the one you prepared as part of Laboratory 8. How consistent were your results? (2 points)

6. Show your calculations of the protein concentration of each pooled fraction. Add as many sections as necessary. The final concentration should be given in **mg/ml** (1 point each, 6 points total)

D65P:

_____:

_____:

_____:

_____:

_____:

_____:

<u>fraction</u>	<u>volume (ml)</u>	<u>LDH activity total ($\mu\text{mol}/\text{min ml}$) units</u>	<u>recovery (percent)</u>	<u>protein (mg/ml)</u>	<u>specific activity (units/mg)</u>	<u>fold purification</u>
crude extract	—	—	—	—	—	—
Ammonium Sulfate Fractionation						
40% AS pellet	—	—	—	—	—	—
40% AS supernatant	—	—	—	—	—	—
65% AS pellet	—	—	—	—	—	—
65% AS supernatant	—	—	—	—	—	—
dialyzed 65% AS pellet	—	—	—	—	—	—

**DEAE-cellulose
column**

IE wash pool A

IE wash pool B

IE elution pool A

IE elution pool B

**CM-cellulose
column**

IE wash pool A

IE wash pool B

IE elution pool A

IE elution pool B

**Cibacron Blue
Column**

AC pool