

EXAM 3: Form B
153L Fall 2005

Name: ANSWER KEY
TA: _____ Section: _____

For TA use:

Q.1. _____ + Q.2. _____ + Q.3. _____ = Total Score: _____

Write your name on each page.

Question 1: (34 points)

You have just finished the first steps in an enzyme purification procedure with the following results:

1. Your initial 27.0 ml crude extract was diluted using a 7 fold three step serial dilution.
2. The third dilution step of the crude extract gave an initial velocity absorbance change of 0.181 min^{-1} , and the first dilution step gave a protein concentration of 31.0 mg/ml
3. The same serial dilutions of the crude extract were used on the 35.0 ml supernatant of the 1st step of a two-step ammonium sulfate fractionation.
4. The third dilution step gave an initial velocity absorbance change of 0.133 min^{-1} , and the first dilution step gave a protein concentration of 21.0 mg/ml.

Note: Do not assume the enzyme is LDH, and you do not need the absorptivity values or exact substrate tube volumes to solve this problem.

Determine the percent yield and purification factor of the 1st ammonium sulfate step?

Just as in lab, the initial data you obtain for the enzyme assay is the $\Delta A/\Delta t$. Since yield a comparison of one step to another, the absorbance change values could be directly compared without converting the values to units/ml, however, the yield is not a comparison of concentrations but of total amounts of enzyme from step to another. In order to obtain the total amounts, you must multiply the concentration by the total volume of the step. The dilution factors are also not issue for the percent yield for they are the same for the crude and the ammonium sulfate step. In this case, you should multiply the absorbance change by the total volume since you will be skipping the conversion step and dilution factors. See below. Notice how it makes it easy for you to assess yield in lab.

% Yield: Units of 1st ammonium sulfate step/ units of crude x 100%

Substitute with absorbance time volume:

$$(0.133 \text{ min}^{-1} \times 35.0 \text{ ml}) / (0.181 \text{ min}^{-1} \times 27.0 \text{ ml}) \times 100\% = 95.3\% \text{ (19 pts. using this method)}$$

Purification factor: S.A. of 1st ammonium sulfate step/ S.A. of crude

For specific activity, total volumes are not important for this value since volume gets cancelled out. In addition, since the dilution factors are for the crude and 40% step, the also need not be accounted for since they will get cancelled out as well.

So the quickest way to determine this value:

$$(0.133 \text{ min}^{-1}/21 \text{ mg/ml}) / (0.181 \text{ min}^{-1}/31 \text{ mg/ml}) = 1.08 \text{ (15 points using this method)}$$

If you were to use the "long method":

- 8 points using absorbance per time as units/ml without the absorptivity. 5 pts. Purification factor (Strategy, Math, and Sig. Figs.)
- 6 pts for multiplying A/t by total volume (correct math included)
- 4 pts. for use or no use of dilution factors
- 5 pts. for percent yield (Strategy, Math, and Sig. Figs.)
- 6 pts. for handling protein mass correctly for specific activity

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Question 2: (24 points)

a) Explain the reasoning why using the same dilution factors as the crude extract for the assays of the 1st ammonium sulfate step provided reliable data for both enzyme and protein assays in problem 1 and for our own LDH purification project. In your answer, you explain the theoretical rationale for the 1st ammonium sulfate step and you use either the volumes in Question 1 or the relative volume predications for LDH Lab 1 to completely explain your answer.

The purpose of the 1st ammonium sulfate step of two-step procedure is to remove contaminants of lower solubility relative to the range of solubility of the target enzyme. Thus, the target enzyme will predominantly remain in the resultant 40% saturated supernatant. (3 points) Proteins also do not precipitate as much at this step (but low soluble contaminants such as fats and lipids do. So there is no change in the amount of protein. (3pts.)
The volume of the supernatant also does not increase (by the addition of salt) significantly. See volumes in problem 1 or your protocol estimations for LDH Lab 1. (3 pts.)

b) Why will you be unable to conclude the exact efficiency of the ammonium sulfate steps based on the yield or purification factor alone? Fully explain which monitoring assay is causing this deficiency and which components of the crude extract is causing this defect.

The ammonium sulfate procedure removes contaminants based on solubility. Some of these contaminants are non-protein contaminants such as left over fats & lipids, nucleic acids, sugars, and even the ammonium sulfate itself. (4 pts.)
The protein assay does not detect other macromolecules because the blue form of coomassie only binds to proteins and not these other type of molecules. The purification factor does not give the actual purification increase from these other contaminants, and the loss in enzyme (% yield) cannot be fully evaluated without know the benefit of purification for this step. (4 pts.)

Without the salting out step – one could not further purify LDH using the affinity column for the column would be clogged by the fats and lipid, and the nucleic acids, sugars, and salts lower LDH binding to the column. (No required for answer).

c) Our LDH purification procedure has two steps: 40% and 60% step. What is the purpose of the 2nd ammonium sulfate step? How exactly is the 2nd ammonium sulfate step prepared?

The purpose of the 2nd ammonium sulfate step is to remove contaminants of higher solubility relative to the range of solubility of the target enzyme. (2 pts.)

The 60% ammonium sulfate pellet is obtained by adding 20% of salt to the 40% supernatant . (2 pts.)

Salt is added slowly (not creating microenvironments of concentrated salt) to a stirred 40% supernatant which is sitting in an ice bath. (2 pts.)

After addition, the 60% saturated solution incubated in the ice bath for 10 minutes and centrifuged. The supernatant is decanted, and the pellets are to be resuspended in buffer. (2 pts.)

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Question 3: (22 points)

You have just prepared a crude extract containing the enzyme R, and you are detecting the presence of enzyme via an activity assay. R catalyzes the following reaction: $X + Y \rightleftharpoons X' + Y'$. You measure the activity by looking at the production of X' (note: X becomes X'). Provide two negative controls you will run on this crude extract that are similar to the ones described in our LDH purification procedure. In your description of the two controls for this experiment, provide each constituent for each control, and if necessary, the dilution of the crude that will be used for the control. Lastly, explain what direct conclusion(s) can be made from the result of each control and the indirect result from both control combined. Write in the spaces provided below.

1st negative control:

constituents:

Reaction mixture contains X, Y and buffer. (4 pts)

direct conclusion(s):

Conversion of X does not occur unless enzyme R is present. (4 pts.)

2nd negative control:

constituents:

Reaction mixture contains X, buffer, and the crude extract (4 pts.) with a dilution that is the same as what is used to measure the enzyme assay. (2 pts.)

direct conclusion(s):

The crude extract does not contain sufficient components that can cause the conversion of X to X' when Y is not present. (4 pts.)

Conclusion from both controls combined:

Enzyme R is required to cause the conversion of X to X', and R can only cause the production via its catalytic activity of Y reacting with X. (4 pts.)