College of the Canyons: Introduction to Biotechnology

Protein Extraction and Concentration Determination Post Lab

1. Referencing the textbook, describe four different techniques to rupture a cell.

1. Mechanical
2. Osmotic (swell to burst)
3. Sound Waves
4)Soap/detergent
5) Enzymes to lyse
6) Boil/heat
7) Freeze
8) Drop (splat)
9) Make it sit through one of Prof. Wolf's lecture..

2. Desribe the roles of the following solutions:

a. Phosphate buffer saline: To wash cells of extracellular proteins (isotonic to avoid cell rupture).

b. Triton X-100 solution: mild soap used to rupture cells (proteins not dissove completely, as some may stay in pellet Esp. if dilute, short incubation, no agitation, etc.)

c. Sodium Dodecyl Sulfate: Solubilizes protein from membrane, imparts a strong negative charge to the protein, making them soluble.

1. Consider the two protein standard curves previosly prepared in lab. Which one is most appropriate for this type of study: unknown in unknown range or unknown in a known range. Defend your answer. Unknown in an unknown range as we are probalby not clear on how much protein is in a cell, solution, etc. With a little bit of math, we may be able to make an educated guess as to the likely protein concentration…so you can see..there is not a single answer to this question…it depends…..

4. Given a protein concentration of 0.18mg per 1ml, and a cell count of 1.97×10^7 cells per ml, determine the protein concentration in mg protein per cell. Use dimensional analysis and show all your work.

$(.18mg/1ml)(1ml/1.97x10^7 cells) = 9.14x10^{-9} mg/cell$

5. Two different types of cells may make identical amounts of proteins, but the concentrations determined using this protocol turn out to be very different. Provide 2 explanations as to these results and do not cite a vague principle (i.e. experimental error). On difference in protein productions in the amount secreted. We washed these proteins away with the PBS washes. Also..SDS theorectically solubilizes membrane bound proteins, but is not equally effective on all cell types. Also, cells may vary on the amount of embedded proteins they have.
6. Review the answers to question number 5, how may some of this "missing protein" may be recovered or otherwise identified? Provide at least one idea relating to this notion. Secreted proteins may be captures by saving the PBS washes and quantify that amount of PBS and protein with in it. Additionally, insoluble proteins may solubilize with additional treatment (like heating, harsher chemicals, more concentrated SDS, etc).
7. The test for proteins we used in lab react with a amino (NH2 /NH3+) and amide groups (N---H). Review the organic macromolecules seen in cells and list three molecues that the test may react with that are NOT proteins. Nucleic acids also contain amide groups. While there are decent amounts of DNA and RNA in a cell, the protein concentration more than swamps these amounts by a few orders of magnitude.