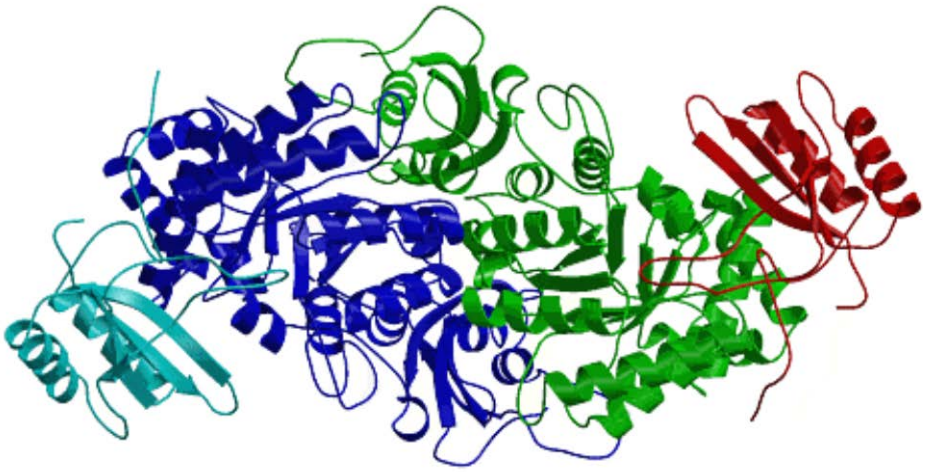
College of the Canyon's Introduction to Biotechnology Course, Custom Lab



Protein Extraction and Concentration Determination

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- **The standard curve that you created in the protein standard curve will be used to determine the protein concentration per cell of your insect cell line.**
- **Prior to determining the protein concentration the cells must be lysed (ruptured) and the protein extracted.**
- **Phosphate buffer saline (PBS) is an isotonic solution that aids in keeping cells viable and intact during preliminary washing phase.**
- **A Triton X-100 lysis solution is used to rupture the cells.**
- **Sodium dodecyl sulfate (SDS) is a strong detergent used to dissolve cell membranes and solubilize cell proteins. Steps including more detergents, protease inhibitors and additional reagents can be used to increase the effectiveness of the process, but have limited impact and or effectiveness.**
- **After the proteins have been extracted from the cell membrane, they are primarily located in the supernatant.**
- **The sample can be assayed using a BCA protein colorimetric test and a multi-well plate reader. Using the absorbance readings, along with your previously prepared protein standard curve, the protein concentration in your sample can be deduced.**
- **Simple calculations will then tell you the protein concentration per cell of your insect cell line. Theoretically, this should be very consistent between student samples, allowing for the accuracy of the processes to be assessed.**

For more information on the College of the Canyons' Introduction to Biotechnology Course, contact Jim Wolf, Professor of Biology/Biotechnology at (661)362-3092 or Email: jim.wolf@canyons.edu

I. Objectives:

1. To become familiar with a protein extraction protocol.
2. To successfully lyse cells from your insect cell line and extract their protein.
3. To use a established protocol for protein detection and a multi-well spectrophotometer to obtain the absorbance 562 nm of your protein extraction samples.
4. Compare resulting protein concentrations by using previously prepared standard curves and to explain rational for various concentration values in different graphs, blanks, replicates and control samples.
5. To obtain the protein concentration per cell of your cell line using your standard curve.

II. Background:

Now that you have created a protein standard curve, you will use it to estimate the protein concentration of the cell line you have cultured. Consider the following ideas, as they will help you in clarifying your work.

- PBS (phosphate buffer saline) is an isotonic solution. It is roughly equal to the saline content of cells so will not rupture or damage cells when added. The buffering helps to keep a stable pH to again aid in keeping cells viable, and also to wash extra-cellular proteins from the intact cells.

- Triton X- 100 is a lysis solution. As the name implies, it aids in lysing or rupturing the cells. It is a weak detergent and can remove proteins if exposure is long enough.

- 10% SDS is a solution of sodium dodecyl sulfate. This is strong detergent and can dissolve cell membranes (as they are made of lipids). Once these membranes are dissolved, they will release their proteins. The SDS also coats the protein. The nonpolar part of SDS will bind with the non-polar portion of proteins and the polar sulfate head will face outward. As a result, nonpolar proteins are easily dissolved in water or another polar solvent

- Through lysing and adding of SDS, most cellular proteins are solubilized and are therefore located in the supernatant (liquid fraction). If a colorimetric assay is then performed on the supernatant, the protein concentration can be deduced.

III. SOP/Lab Activities:

Important: Always note any addenda that are posted by the instructor. Work in pairs, but you will each make your own notebook entry, analysis, graph, etc. Each student will assess their own insect cell line and the team will also work on a common sample

provided by the instructor. ***BE SURE TO CLEARLY LABEL AND TRACK ALL SAMPLES!***

1. Protein Extraction:

1.1 After a cell transfer, save one of your insect cell cultures. Pick a culture that is has a **really high density** of cells to help ensure adequate protein for extraction. Record what cell line this is, and be sure that this is one of the cell samples you did a count on. Also, get a cell sample from the instructor and do the following steps on BOTH lines on cells.

1.2 Sub-aliquot your cell sample into 5 separate microfuge tubes 200µL each (and save remaining cell culture in case a mistake is made later!). Also get 1 ml from the instructor from the "stock" cell cultures at the front of the class. Both you and your partner need 1 ml each. Record what sample you got form the instructor (in your lab notebook). Divide this sample into 5 labeled 1.5 ml microfuge tubes. So, each team should now have 20 tubes: 5 from student A' insect cell line, 5 from student B's insect cell line, **two** sets of 5 from the stock culture.

Please note…you will need to use centrifuges, vortexers, etc…so keep track of everything and do not hesitate to use the devices in the instrument room. The procedure from now on will mention what steps you are doing, but not necessarily remind you to do this to each tube…so keep things straight.

1.3 Add 1ml of Phosphate Buffer Saline (PBS) to each tube, vortex and spill 1000 rpm for five minutes, discard supernatant (pour off into beaker, and try not to dislodge pellet). Repeat this process one more time (1 ml PBS, vortex, spin, pour off supernatant).

1.4 Using 200µL if the Triton 100 X lysis buffer, re-suspend the pelleted cells by vortexing. Keep samples at room temperature for 10 minutes.

1.5 Place in freezer for 10 minutes. A minus 80 ^{o}C freezer will be used. So, get all 20 samples in a rack, label clearly and give to instructor to place in freezer.

TECHNICAL NOTE: Minus 80 ^{o}C freezers are extremely cold. As a result, you do not want to leave them open for any amount of time beyond what is absolutely necessary. Know what you are going to do BEFORE you open the freezer. Open the door carefully, do the work, close the door. The instructor will do this for you the first time, but you should keep this idea in mind, as the freezers are: VERY useful, very sensitive, and you may be using it in the near future.

1.6 Remove samples from freezer and thaw in water bath (about 1-2 minutes), and centrifuge for 15 minutes at top speed (14,000 RPM).

1.7 Using a micropipette, transfer 200µL of the supernatant to a new microfuge tube (again, do not disturb the pellet).

1.8 See instructor to ensure you understand this step/the math. Add 10% SDS to the above solution until a final concentration of 0.2% SDS is attained (Use $C_1V_1=C_2V_2$). For example:

$C_1=0.10$, $C_2=0.002$, and $V_2=200\mu L$ ∴

$(0.10)(V_1)=(0.002)(200)$ $V_1=4\mu L$

1.9 Incubate at 37° for 5 minutes. These will be your samples to assess the protein concentration. To do this, refer to step 4; Developing the Samples in the Protein Standard Curve lab. Recall that 25 µl of sample and 175 ul ml of BCA solution are required per well. A student team can share a plate. In addition to the 20 samples, include 4 blanks (200 ul of BCA). To help remind you of what samples went where, a copy of the 96 well blank template is included on the last page. Copy, cut and paste this into your lab notebook so you can reference as needed.

2. Concentration Determination:

2.1 Use the standard curve that you created in an earlier lab and determine the protein concentration of your sample based on the <u>average</u> ABS reading you obtained. <u>Be sure to show your interpolation of this data on your standard curves.</u> Create a table of protein concentrations from the various graphs that you have in you protein concentration. Some graphs may not be applicable as the ABS reading may be too high or low to apply. Address this issue as needed and discuss in you lab notebook.

2.2 You now know the protein concentration of your cell line in mg/mL. The last step is to determine the protein concentration per cell (mg protein/cell). In order to do this you will have to know the number of cells per mL. You should have completed a cell count on the same cell line you obtained your protein sample from. Now use dimensional analysis to obtain the amount of protein per cell. For example, if you determined that the protein concentration of your cell culture is 0.12mg/mL and you have $1.93x10^7$cells/mL (note: all cells, not just viable) then:

$$\frac{(0.12 \text{ mg protein})(1 \text{ ml})}{(1 \text{ mL})(1.93 \times 10^7 \text{ cells})} = 6.22 \times 10^{-9} \text{ mg protein/cell}$$

IV. <u>Post-Lab Questions/Activities</u>:

The following post lab questions are for your benefit. The questions will help you to address a range of topics relating to the lab activity. Along with the post lab handouts, these questions will help to ensure that you have both correct information regarding the lab data and crucial lab processes. Complete the post lab questions at the end of the lab and post lab handouts (keys for both of these are available from your instructor) before making any lab-notebook entries.

1. What was rational for PBS? Freezing and thawing? SDS?
2. What was left in pellet after last centrifugation? In the supernatant after the first centrifugation?
3. This technique does not get all of the cellular protein. Look at the procedure and list a few ways that cell protein may not have made it into the final assayed fraction (the part with the 0.2 % SDS.)
4. Was the blank as complete as it could be? Why or why not?
5. Consider the values of proteins generated from the data. Cite at least three factors that could explain some of the variability in the samples.

V. **Notebook Entries:** Data from the lab should be the focus of this section and if there are any incorrect results, you should discuss this as well as expected results. Section V will contain both your results and discussion. Your data should drive the discussion. An informed discussion is dependent on understanding the post lab questions/activities.

Your intro should:

- I.D this as a lab on protein quantification of proteins in *D. melanogaster* cells.
- Cell counts along with detergents and spectrophotometric analysis allows for gross quantification of protein.
- Allude to previously lab activities and impact (i.e. protein standard curve).

Results should be:

- Copy (Xerox) appropriate curves from earlier protein standard curve.
- Use of previously prepared protein standard curves to show protein amount (from 2 or 4 curves), counts of cells, and example calculations to mg of protein per cell. A table of data may be useful.

Discussion should consider the following:

- Compare results with colleagues and see if values are within a close range.
- Give 3-4 reasons for range (**be specific**). Try to keep brief (2 paragraphs maximum).