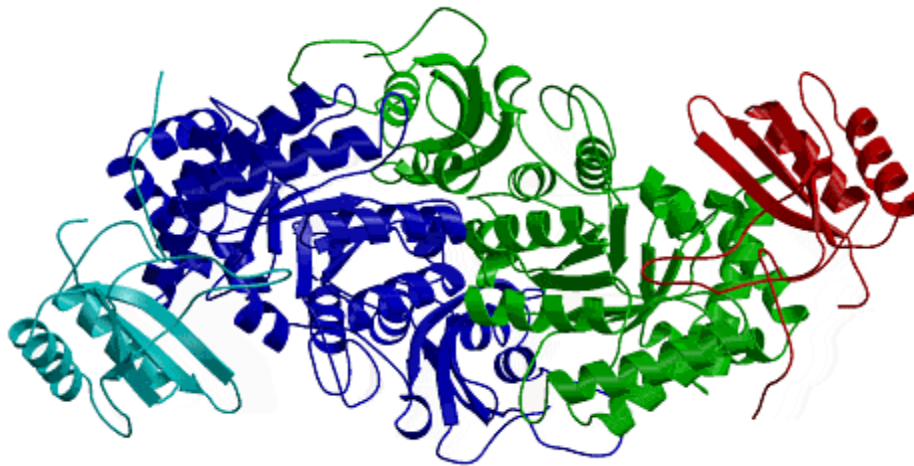


COC Biotechnology Program



Protein Extraction and Concentration Determination

Version 12-03-03

- 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.
- 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.
- Once the protein has been extracted from the cell it is located in the supernatant.
- Finally the sample can be assayed using a spectrophotometer and the ABS reading, along with your standard protein curve, used to determine the protein concentration in your sample.
- Basic calculations will then tell you the protein concentration per cell of your insect cell line.

For more information on the College of the Canyons Biotechnology program contact Jim Wolf, Associate 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 spectrophotometer to obtain the ABS 562 of your protein extraction.
4. 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.
- 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.

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 200 μ L each (and save remaining cell culture in case a mistake is made later!) These are the tubes that you will be using for the protein visualization so be sure and use the 2 ml round bottom tubes (recall last lab).

- 1.3 Add 1ml of Phosphate Buffer Saline (PBS) to each tube, vortex and spin @1000 rpm for five minutes, discard supernatant (pour off into beaker, and try not to dislodge pellet). Repeat this process one more time.
- 1.4 Using 200 μ L of the Triton 100 X lysis buffer, resuspend the pelleted cells by vortexing.
- 1.5 Place in freezer for 10 minutes, then in water bath (long enough to thaw), and centrifuge for 15 minutes at top speed.
- 1.6 Using a micropipette, transfer 200 μ L of the supernatant to a new microfuge tube (again, do not disturb the pellet).
- 1.7 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:

$$C_1=0.10, C_2=0.002, \text{ and } V_2=200\mu\text{L} \therefore \\ (0.10)(V_1)=(0.002)(200) \quad V_1=4\mu\text{L}$$

- 1.8 Incubate at 37° for 5 minutes. This will be your sample to assess the protein concentration. To do this, refer to step 2; Develop the Samples (2.1.&2.2) in the Protein Standard Curve lab. Recall that 100 μ l of sample and 1.9 ml of BCA solution are required.

2. ABS 562 Determination:

- 2.1 Blank the spectrophotometer with PBS.
- 2.2 Collect the ABS data for all five of your protein samples. Remember to use the plastic curvettes and record all data in your lab notebook.
- 2.3 Obtain an average ABS reading for your protein samples.

3. Concentration Determination:

- 3.1 Use the standard curve that you created in the last lab and determine the protein concentration of your sample based on the average ABS reading you obtained. Be sure to show your interpolation of this data on your standard curve.
- 3.2 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 1.93×10^7 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. Post-Lab Questions/Activities: 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 washes? Freezing and thawing?
2. How much would your results be different if twice the amount of Triton 100 X was used? Twice the PBS?

3. What was left in pellet after last centrifugation? In the supernatant after the first centrifugation?
4. 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.)

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:

- First step in analysis is to isolate *D. melanogaster* as source of cells.
- Counts along with detergents and spectrophotometric analysis allows for gross quantification.
- Comparison of X/mg/cell will allow review of technique.

Results should be:

- Use of previously prepared protein standard curve to show protein value, counts of cells, and calculations to mg of protein per cell.

Discussion should consider the following:

- Compare results with colleagues and see if in range.
- Give 3-4 reasons for range (be specific).

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