

## Protein Extraction Protocol

Now that you have created a protein standard curve, you will use it to estimate the protein concentration of your cell line you have cultured. Since today is Monday, prior to doing a cell transfer, take 1 ml of your sample to assay. Start the below protocol soon, and remember to use the down time to do another cell transfer and count (viable and not viable.)

Sub-aliquot your cell sample into 5 separate microfuge tubes of 200 ul each.

Add 1 ml of Phosphate Buffered Saline (PBS) to each tube, vortex, spin @ 1000 rpm for five minutes, discard supernatant (pour off into beaker, and try not to dislodge or disturb pellet.) Repeat this process two more times.

Using 200 ul of the Triton 100 X lysis buffer, re-suspend the pelleted cells by vortexing.

Place in freezer for 10 minutes, thaw in water bath and centrifuge for 15 minutes at top speed.

Using a micropipet, transfer the 200 ul of supernatant to a new microfuge tube.

Add 10 % SDS to the above solution until final concentration of SDS is 0.2 % is obtained.

Using the previous protein protocol get a spec. reading for your cells. If your samples are too concentrated (i.e. they are way off the

See other handout for using standard curve data to calculator

What was rational for freezing and thawing.

How would your results be different if twice the amount of Triton 100 x lysis buffer were used. Twice the PBS?

What was left in the pellet when you centrifuged.