

## HANDOUT 4: Protein Extraction from Cell Culture

Protein extraction can be cell type specific. Thus, reviewing the literature for your cell type or field is a good place to start when choosing a method for protein extraction. Because there are many options for buffer exchanging samples to remove MS interfering reagents, it is always best to extract proteins in the most biologically relevant manner than considering methods only for MS compatibility. There is likely no reason to change the current protocol you are using to study your samples, especially if your sample preparation is well-characterized in your lab or yields the specific phenotype. A brief review of your sample preparation details will determine if any changes would benefit the project goals and MS analysis. However, if you do not have a sample preparation preference, we have had success with labs that use the following protocol to extract proteins from cell cultures for MS analysis. We highly recommend using SDS-PAGE to monitor your protein extraction efficiency and reproducibility.

Please note that if you choose a protocol that used urea, urea can carbamylate proteins if the urea is low quality, heated above 25°C or stored for more than 2 weeks. For urea containing methods please only use the highest ACS quality urea and AG 501-X8 mixed bed resin (w/ color indicator, BioRad) to remove the carbamates from urea. In addition, if you prefer, you may choose other protocols which use detergents, hypotonic solutions or grinding in liquid N<sub>2</sub> for extracting proteins.

### Materials:

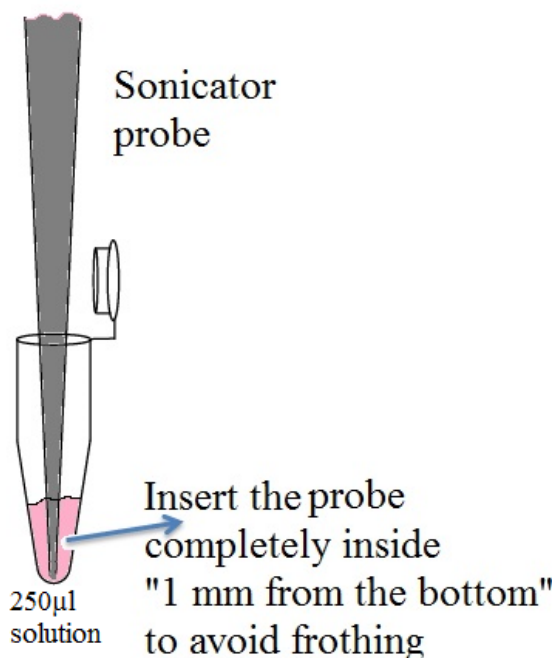
Phosphate buffered saline (PBS), ice cold  
100mM PMSF stock in isopropanol  
2% SDS containing 1 mM EDTA and 1 mM PMSF  
Water, HPLC Grade, Fisher  
Probe Sonicator (Micro)  
Eppendorf Centrifuge  
For phosphoproteomics, add these phosphatase inhibitors  
    1 mM NaOrthovanadate,  
    1 mM NaPyrophosphate,  
    1 mM Beta glycerophosphate  
    1 mM NaF

### Notes:

1. SDS will precipitate out of solution at 4°C or below.
2. After lysing cells in 2% SDS with protease inhibitors the sample is stable at room temperature
3. Sonicate on ice in bursts to prevent overheating samples
4. DO NOT use tubes advertised as “low protein binding”

**Protocol:**

1. Wash the cells with ice cold phosphate buffered saline (PBS) 5 times to remove serum proteins. Maintain the temperature at 4°C during washes.
2. For 10 cm size dish, scrap the cells using 0.5 ml 2% SDS containing 1 mM EDTA and 1 mM PMSF (phenylmethyl sulfonyl fluoride, freshly prepared from a PMSF stock in isopropanol).
3. If phosphoproteome is desired then with SDS buffer include 1 mM NaOrthovanadate, 1 mM NaPyrophosphate, 1 mM Beta glycerophosphate and 1 mM NaF.
4. Sonicate on ice as shown in figure. 20% power input sonicate for 5 sec, give 10 sec gap repeat 20 times or until the cloudy cell lysate become clear.
5. Centrifuge to remove any pellet. Store at -80°C.
6. Dilute small portion of sample and measure total protein concentration using micro BCA or other reagent compatible protein assay.
7. Use SDS-PAGE to assess protein extraction efficiency and reproducibility.
8. Aliquot sample into 50 or 100 ug total protein per eppendoff tube.
9. Store protein aliquots (e.g. 100 ug) at -80°C



**If you use Urea****Make 9M urea (use within 1-2 weeks)**

1. Weigh out urea (highest ACS grade, Sigma)
2. Add half the volume of solution you ultimately want  
(example: 500 mL total of urea, add 250 mL to the weighed-out urea)
3. Stir on hot plate (set to 25°C) for 1-2 hours until urea goes into solution  
**DO NOT LET UREA GET OVER 30 DEGREES!**
4. Wash 30 ml of dry AG 501-X8 mixed bed resin two times with HPLC grade or distilled water.
5. Add resin slurry to urea solution
6. Stir urea-resin solution for 30-60 minutes
7. Filter urea in 250 mL media glass filter flask
8. Bring urea volume up to a final concentration of 9M
9. Store in glass bottle at room temperature  
**DO NOT REFRIGERATE, UREA WILL PRECIPITATE.**
10. Use within 1-2 weeks.