

HANDOUT 3: Protein Extraction from Tissue

Protein extraction can be tissue or cellular compartment specific. Thus, reviewing the literature for your tissue 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 tissue for MS analysis. We highly recommend using SDS-PAGE to monitor your protein extraction efficiency and reproducibility.

Please note that this protocol uses **urea** which can [carbamylate](#) proteins if the urea is low quality, heated above 25°C or stored for more than 2 weeks. This protocol includes steps to reduce or eliminate the carbamates from urea. In addition, although this protocol omits the use of any detergents, if you prefer, you may choose other protocols which use detergents, hypotonic solutions or grinding in liquid N₂ for extracting proteins.

Materials:

AG 501-X8 mixed bed resin (w/ color indicator), BioRad
Urea, highest ACS grade, Sigma
Water, HPLC Grade, Fisher
Methanol, ACS Grade, Fisher
Chloroform, ACS Grade, Fisher
1M HEPES stock, Quality Biologicals
1M Triethylammonium bicarbonate (TEAB), pH 8.5, Sigma
250 mL media glass filter flask
Powergen 125 homogenizer from Thermofisher
Vortex
Eppendorf Centrifuge
Speedvac

Protocol:

A. 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.

B. Tissue Homogenization

1. Add 5 volumes of 9M urea in 10 mM Hepes, pH7.3
2. Homogenize using a Powergen 125 at room temperature ramped up to power setting #6 for approximately 10s. Avoid frothing.
3. If necessary, let sit for 30 mins at room temperature

C. Methanol chloroform extraction

1. Dispense Methanol, chloroform and water into glass storage jars
DO NOT USE POLYPROPYLENE OR POLYSTYRENE TUBES to avoid leaching plasticizers, etc.
2. Add 0.4 ml of methanol per 0.1 ml of protein extract
3. Vortex
4. Centrifuge at 9,000g for 10 sec
5. Add 0.1 mL of chloroform per 0.1 ml of original protein extract
6. Vortex
7. Centrifuge at 9,000g for 10 sec
8. Add 0.3 mL of distilled H₂O to per 0.1 ml of protein extract
9. Vortex vigorously
10. Centrifuge at 9,000g for 1 min
11. Discard upper aqueous layer of supernatant
(Protein will be between the two layers)
12. Add 0.3 mL of methanol per 0.1 ml of original protein extract
13. Vortex then centrifuge at 9,000g for 2 min
14. Remove supernatant
15. Vacuum centrifuge (Speed vac) to dryness
16. Resuspend in less than 50 ul 100 mM TEAB, 1 mM DTT.
(If proteins do not resuspend you may add up to 1% SDS.)
17. Quantify total protein concentration using micro BCA or other reagent compatible protein assay.
18. Use SDS-PAGE to assess protein extraction efficiency and reproducibility.
19. Aliquot sample into 50 or 100 ug total protein per eppendoff tube
DO NOT USE TUBES ADVERTISED AS "LOW PROTEIN BINDING"
20. Store protein aliquots (e.g. 100 ug) at -80°C preferably dry