

## TISSUE EXTRACTION METHODS:

### Important points to consider:

- 1) pH: the pH at which the protein of interest may be stable may not be the pH at which it will be active. The pH of buffers can also affect the activity of known proteases.
- 2) Salts: solubility can be increased with a moderate amt of salts. This may affect the ability of the proteins to bind to chips or may lead to adducts
- 3) Detergents: use of non-ionic detergents at concentrations below known CMC is important for protein profiling applications. Use of ANY charged detergents such as SDS will interfere with the TOF-MS and be technically difficult to see any peaks.

### Buffers Used in Protein Work:

| Buffer         | pKa               | Properties    |
|----------------|-------------------|---------------|
| NaAcetate      | 4.75              |               |
| Nabarbonate    | 6.50; 10.25       |               |
| Nacitrate      | 3.09; 4.75; 5.41  | binds Calcium |
| NH4acetate     | 4.75; 9.25        | volatile      |
| NH4bicarbonate | 6.50; 9.25; 10.25 | volatile      |
| Tris-chloride  | 8.21              |               |
| Naphosphate    | 1.5; 7.5; 12.0    |               |
| Tris-phosphate | 7.5; 8.21         |               |

### Detergents

| Name            | Ionic   | CMC (%w/v) | Mwt of micelle |
|-----------------|---------|------------|----------------|
| TritonX-100     | non     | 0.15       | 90kD           |
| NP-40           | non     | 0.023      |                |
| Octyl glucoside | non     | 0.73       | 8kD            |
| Tween-80        | non     | .0015      | 76kD           |
| CHAPS           | zwitter | 0.49       | 6kD            |
| Nadeoxycholate  | anionic | 0.21       | 4kD            |
| SDS             | anionic | 0.23       | 18kD           |

### Important points to consider:

- \* Ionic detergents interfere with SELDI/TOF-MS and are NOT recommended.
- \*DEPC water is NOT recommended
- \*Glycerol is NOT recommended
- \*If you are going to use reducing agents such as DTT or bme, your sample will have to be considerably diluted in order to study

**Tissue Extraction:** (does not include subcellular fractionation or nuclear fractionation).

**Method 1:**

**Applicable to soluble cytoplasmic proteins**

**LYSIS BUFFER:**

25mM Hepes, pH 7.4, 50mM NaCl, 1mM EDTA, 0.1mM PMSF + protease inhibitors

Perform dounce homogenization or sonication. Keep samples chilled. Strokes of hand homogenization should be monitored by microscopic inspection.

**Method 2:**

**LYSIS BUFFER:**

25mM Hepes pH 7.4, 50mM NaCl, 1% NP-40, 0.1 mM PMSF + protease inhibitors

Lyses buffer should be cold at all times

Add to pellet (about 200ul/4x10<sup>6</sup> cells), pipet up and down to disaggregate, incubate on ice for 10 min and spin 10 min in microfuge at highest rpm. Save supernatant.

**Method 3:**

**Mechanical Disruption Techniques:**

**LYSIS BUFFER**

Use either 0.1xPBS or ddH<sub>2</sub>O

Lyses buffer should be cold at all times

Add equivolume to cell pellet and draw sample up into a small bore needle, do this several times until microscopic inspection shows cellular disruption. Spin the cells to pellet insoluble membranes. This technique will only solubilize hydrophilic proteins. In order to solubilize membranes, following mechanical disruption add 0.1-0.5% nonionic detergent, then centrifuge to remove insoluble material.

**Method 4:**

**LYSIS BUFFER: this is the standard 2D gel extraction method**

9M Urea, 2%CHAPS, and protease inhibitors

Buffer can be stored at -20C but must be aliquoted. No freeze/thawing!

Keep at RT due to crystallization of urea at low temps.

With a strongly denaturing buffer, there should be little to no protease activity. As with any lyses technique a mechanical type of disruption can be added, such as sonication or needle/syringe cell breakage (detergent will cause bubbling and this should be avoided)

For membrane fractionation:

Use method of Thom et al Biochem 1977: this method is an easy way to separate plasma membrane proteins from the rest of the cell membranes.