

## **Protein and Peptide Analysis by Electrospray (ESI) Mass Spectrometry and MALDI-TOF Mass Spectrometry**

### **Sample Preparation for molecular weight determination of intact proteins**

#### **Electrospray (ESI) Mass Spectrometry:**

##### **General sample handling**

To minimize protein losses from adsorption to walls of tubes, use polypropylene tubes or silicized polypropylene tubes. Avoid polystyrene (clear plastic) and glass.

Use freshly prepared, high purity reagents and water. Contaminants from buffers, detergents, lipids can prevent a good mass spectrometry result.

##### **Sample submission**

- ◆ In solution, as concentrated as possible
- ◆ Minimum volume 20-50 microliter
- ◆ Minimum Concentrations:

Peptides/ (500-5000 Da):	1 pmol/ microliter
Proteins 5- 20kD :	5 pmol/ microliter
Proteins 20- 66kD:	20 pmol /microliter
Proteins 67-100kD:	40 pmol/microliter

Evidence of concentration determination must be provided upon submission.

##### **The sample should NOT contain at all**

Azide	SDS
Brij 35	Tris base
CHAPS	Triton X-100, reduced Triton X-100
DMSO	Trifluoroacetic acid
DMF	Tween
Glycerol	Zwittergent
Phosphate buffers	any other detergent
Salts and buffers >100 mM	

##### **The following components and solvents are ACCEPTABLE**

Acetic acid  
Formic acid  
Aqueous ammonium hydroxide  
Acetonitrile  
Methanol, Ethanol or Isopropanol  
Guanidine/HCl up to 4M  
Salts and non-phosphate buffers < 100 mM

## **MALDI-TOF Mass Spectrometry:**

### **General sample handling**

To minimize protein losses from adsorption to walls of tubes, use polypropylene tubes or silicized polypropylene tubes. Avoid polystyrene (clear plastic) and glass.

Use freshly prepared, high purity reagents and water. Contaminants from buffers, detergents, lipids can prevent a good mass spectrometry result.

### **Sample submission**

- ◆ In solution, as concentrated as possible, volume 10-20 ul.
- ◆ Minimum Concentration 10 pmol/microliter
- ◆ Use 200ul PCR-style microcentrifuge tubes

### **The sample should NOT contain at all**

Azide	SDS
Brij 35	Tris base
CHAPS	Triton X-100, reduced Triton X-100
DMSO	Tween
DMF	Zwittergent
Glycerol	any other detergent
Phosphate buffers	
Salts and buffers >100 mM	

### **The following components and solvents are ACCEPTABLE**

Acetic or formic acid  
Trifluoroacetic acid  
Acetonitrile, ethanol  
Guanidine/HCl 4M  
Hexafluoroisopropanol less than 40%  
Methanol  
Urea (<50 mM)  
Salts and non-phosphate buffers < 100 mM