



## Mass Spectrometry Sample Preparation Guidelines

**NOTE:** Keratin contamination is a significant problem when analysing samples by MS. Gloves should be worn and all care should be taken to ensure keratin is not introduced while handling samples.

### Samples submitted in gel band or gel spot form

- **Reduction and Alkylation** - If you are to submit samples in gel form for MALDI MS or 1D nanoLC ESI MS/MS for protein identification please specify chemicals you have used for reduction and alkylation. If your sample has not been subjected to reduction and alkylation we will have to do in-gel reduction and alkylation to your sample for efficient trypsin in-gel-digestion and will charge the service fee accordingly.

The following are two protocols for reduction and Alkylation that you may use to your sample prior to gel electrophoresis separation:

#### Protocol 1, using TBP and acrylamide for reduction and alkylation

1. Dissolve protein sample in 7M Urea, 2M Thiourea, 20mM Tris, 4% Chaps at a concentration of about 1 to 5 mg/ml.
2. Add reducing reagent TBP into the sample solution to make TBP concentration 5mM.
3. Add alkylation reagent acrylamide into the same solution to make acrylamide concentration 10mM.
4. Incubate at room temperature for 60 – 90 minutes.

#### Protocol 2, using DTT and iodoacetamide for reduction and alkylation

1. Dissolve protein sample in 7M Urea, 2M Thiourea, 20mM Tris, 4% Chaps at concentration of about 1 to 5 mg/ml.
2. Add DTT into sample to make the DTT concentration of 100mM, incubate for 60 minutes at room temperature.
3. Add Iodoacetamide into the sample solution to make the concentration to be 15mM, incubate for 60 minutes at room temperature in the dark.

- **Gel Staining** – We recommend CBB G250, R250, Sypro Ruby or Deep Purple staining . If you must use silver stain, we recommend using one of the following:

1. Shevchenko A, Wilm M, Vorm O, Mann M: *Mass spectrometric sequencing of proteins from silver stained polyacrylamide gels*. Analytical Chemistry (1996) 68(5):850-858.
2. Blum H, Beier H, Gross HJ: *Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels*. Electrophoresis (1987) 8:93-99.

### 3. BioRad Silver Stain Plus Kit.

- **Gel cutting** – Gel band or gel spot should be cut carefully to minimise gel area that does not contain protein. Large gel piece should be further sliced into about 1 mm<sup>3</sup> sizes for good in-gel-digestion.
- **Sample vials** – Gel pieces should be excised and placed in eppendorf tubes. Each sample vial should be labelled clearly and properly sealed.
- **Posting** – Gel samples can be posted in room temperature.

#### **Samples submitted in Solid form**

- Salt and detergent free
- Samples for intact protein mass analysis must be of high purity (>90%) and should be of no less than 100 microgram in quantity.
- Sample vials should be clearly labelled and sealed.
- Solid samples can be posted in room temperature.

#### **Samples submitted in liquid form**

- As proteins are not stable in solution, this is not the ideal form to post protein samples. If you have to send samples in solution, the sample should be stored in freezer and shipped on dry ice. Please inform APAF the posting of the sample so that we will store the sample in freezer immediately upon receiving.
- For intact protein mass analysis, the concentration of the protein should not be less than 5 picomole per microliter and about 20 microlitre or more should be submitted.
- The salt concentration must be less than 5 mM and the detergent must be removed. All known chemicals in the solution should be listed in the service Request Form.
- Sample vials should be clearly labelled and sealed.

#### **Pathogenicity**

Biological samples coming into APAF should be accompanied by documentation of potential pathogenicity or pathogen free status otherwise APAF will presume all samples from human and animal origin are potential pathogens and will be treated accordingly.

#### **How do I send samples from Overseas to APAF?**

For guidelines and documentation required visit our [Quarantine guidelines](#).