Sample Preparation for Protein Identification

Sample preparation is the most important aspect of any analytical technique. Protein identification by mass spectrometry requires enough sample for signal detection and little background from contaminating peptides e.g. keratin from skin and hair; bacterial growth in old solutions. Samples for mass spectrometry must be prepared in the cleanest environment possible.

Common sources of contamination:

- Poorly handled solutions of acrylamide, tris, glycine, and SDS sample buffer,
- Improperly cleaned glass plates
- Dirty glass dishes used for staining
- Dusty micro-centrifuge tubes or tips

To avoid contamination:

- Use a clean (acid washed) glass tray to stain the gel and do not touch the gel with your hands
- Always wear gloves
- Rinse the gloves with water to remove talcum powder and dust
- Frequently replace your gloves to reduce sample contamination by skin, dust, hair etc.
- Mishandling of gel samples is very common, and leads to useless results for a lot of labor
- Please contact the facility for advice prior to your first attempt at sample prep
- It is always a good idea to supply an identically treated negative control band/spot to help eliminate potential artifact peaks

Please note:

- Not all silver staining protocols are compatible with mass spectrometry
- Silver-stain kits and protocols that contain glutaraldehyde in the fixing solution must be avoided, as glutaraldehyde cross-links proteins and the digestion will result in improper peptides
- Extensive washing steps preceding reduction and S-alkylation can be omitted if the silver staining technique is used.

- Coomassie Brilliant Blue stain is also amenable to protein identification by mass spectrometry provided that your protein is in a sufficiently high concentration for detection by this technique
- Other staining procedures that should work for MALDI-TOF:
    - Sypro Ruby Red
    - Sypro Orange
    - negative zinc or copper

Important:

1. For proteins less than 30 kDa the use of higher percentage gels (approximately 15%) is recommended to minimize loss of sample during staining/destaining procedures
2. For in-gel trypsin digestion and elution of peptides, protein samples should be in the smallest gel volume possible
3. If possible use 0.5 to 0.75 mm thick gels and keep the protein sample in a single gel line such that the final density of the protein in the stained gel will be at least 0.05 mg/mm3