Mass spectrometry Facility

Protein/ Peptide Sample Submission Guide

Protein gel sample Guideline:

It is **very important** that the whole gel sample preparation is done in a keratin-free environment. Always wear latex gloves (and change them frequently). Work in a laminar flow hood when possible.

Use freshly cast gels in order to avoid degradation of polyacrylamide. Make sure that the gels are polymerized completely (polymerization time, fresh temed and ammonium persulfate) and used as soon as possible. If you use precast gels from any of the major suppliers, it is the best that they are used within two weeks of the production date (usual shelf life is 3 month by manufacturer's definition).

Visualization of protein spots/bands should be done by mass spectrometry friendly dyes. Silver staining protocols should not contain any gluteraldehyde. Keep in mind that the detection sensitivity of mass spectrometers generally is not as high as the gel visualization dyes. For example, silver and fluorescent staining can detect as low as 1 ng protein from a gel band. However, mass spectrometers are not able to detect such small amounts from gels. In order to obtain quality mass spectra, a sufficient amount of protein is very important. We suggest that you submit 50-200 ng/protein for the purpose of identification and >200 ng/protein for high sequence-coverage or modification identification purposes.

Gel bands/spots for mass spectrometry analysis should be excised as soon as possible. If you can't excise the spots immediately, store the gels in the refrigerator. In order to avoid keratin contamination, always wear gloves and use a clean workbench, clean pipette tips, blades, tubes and solvents when cutting the gels. If possible work in a laminar flow hood. Try to cut the bands/spots as accurately as possible, since the excess unstained gel will increase the detection of background components and suppress the ions of interest in mass spectrometry analysis. The gel pieces need to be stored in a -80°C freezer if not shipped right away.

Shipping of the gel samples should be done under dry-ice conditions if shipped from outside Edmonton. Higher temperatures will likely cause polyacrylamide degradation and result in reaction with proteins in the gel. This will affect the enzymatic digestion and extraction of peptides from the gel.

Protein/Peptide Solution Sample Guideline:

Buffers in the sample should be minimal. If it is necessary to store samples in buffer, clean-up procedures prior to digestion or analysis may be necessary; this will likely introduce sample loss and prolong the analysis process.