

## UMKC Mass Spectrometry and Proteomics Laboratory Guidelines for Submitting Samples for Mass Spectrometry Analysis

A sample submission form is available online ([http://sbs.umkc.edu/research\\_proteomics.cfm](http://sbs.umkc.edu/research_proteomics.cfm)). Please bring this form to the lab with your samples. Sample information entered on this form will include the choice of analysis you need and the type of sample(s) you are submitting. Please identify the species of sample for best protein identification results. This is because many peptides are conserved among species and/or protein isoforms among species, potentially making identifications ambiguous without this information. If gel bands may have recombinant protein with novel affinity tag, fusion/chimera, or any sequence not present in national database (NCBI), email the sequence (single letter code) of your translated reading frame. If you are bringing a gel, your gel will be scanned in the Facility for our records, and to include with the report. You may provide a photo with bands marked, or identify them in person when you bring the gel. Each separate sample will receive a unique number along with the information you provide, in the Facility Sample log book. This documentation is maintained for sample tracking, and sample names are included in the MS data files, providing an unambiguous link to the correct sample.

Samples can be submitted either in solution or resolved in acrylamide gels (typically SDS-PAGE). Recommendation for gel staining is described below. Precipitated or blotted samples are not recommended because of solubility problems (PVDF or other western blots do not release peptides for analysis), but we can attempt to extract them if blotted onto Whatman paper. Dried gels can yield peptides for analysis, but typically with lower abundance.

For MALDI analysis, samples may be submitted in solution (a reversed phase LC fraction, for example). Alternatively, a dried sample can be submitted. Protein and peptide samples in solution should be submitted in an aqueous solution, with minimal salt content and minimal organic solvent. If you are submitting an organic compound, you should probably bring a small amount of solvent that you know dissolves the compound (THF, Chloroform, toluene, for example). Please include an estimate of solvent content and salt content on the sample form.

If your samples have been resolved by SDS-PAGE (including 2D gels), the following gel preparation is recommended. Separate the plates, and place the gel in a fixative solution containing 50% Ethanol and 10% acetic acid (methanol will also work in place of ethanol, but it is significantly more poisonous). Do not use fixative solutions that contain an aldehyde. Gently rock the gel in the fixative for 30-40 minutes. Change to good deionized water, and wash for 30 minutes. Replace the water once, and allow it to rinse for another 30 minutes. Now, stain your gel with a colloidal coomassie blue such as Pierce's GelCode Blue, or BioRad's BioSafe staining reagents. You can allow these to stain overnight, gently rocking, and then wash out the excess with water. Destain fairly completely, because it will improve analysis, and the band just needs to be seen for excision. If destaining is necessary, you can wash the gel in 50% ethanol, with 10% acetic acid. When you have achieved good contrast, switch to water for two full washes of at least 30 minutes each. Gels need to be submitted in water. For shipping, a Seal-a-Meal type of pouch (or Ziploc bag) will keep it from drying during transportation. We have a scanner available for gel documentation in room C418. If we scan the gel for you, there is no fee, and a gel image with bands marked for excision will be sent to you for confirmation before we start.