Sample Preparation and FAQ´S

- Some chemicals, namely not easily volatilizable compounds, such as salts, detergents or some buffers, may contaminate the mass spectrometer. In consequence, the equipment sensitivity is strongly reduced, which will compromise the quality of the experimental results and avoid carrying out other studies. The decontamination process is usually time consuming (around 2 weeks). Sponsors should be aware of this fact in order to avoid the presence of potential contaminants in their samples. Doubts about sample preparation or composition should be presented to the Mass Spectrometry Unit (UniMS) Coordinator (unims.direction@itqb.unl.pt).

Q: What sort of sample is accepted?
A: Polyacrylamide gel pieces containing the protein of interest are accepted, from both 2D- and 1D SDS-PAGE gels. Please note that on 1D gels protein-band overlap often occurs. For LC-MS analysis, only known amounts and precipitated protein samples are accepted.

Q: Do I need to see a band for my protein of interest, in gel?
A: Yes, this is the most important prerequisite. If you do not see a definite band by some protein detection method, you probably won't get any useful result.

Q: What is the sensitivity? How much protein do I need?
A: For "in-gel analysis", the more, the better! If you want to have a good result you should plan to load on your gel about 2.0 pmol of the protein you want to analyze. For a polypeptide of 50 kDa this corresponds to about 0.1 µg. It is possible to obtain results with much less (down to 100 fmol starting material) but then the nature of the protein tends to play a major role. Some proteins just digest and are recovered much better than others. Remember, it is worth investing a bit more work to prepare 2-3 times more protein than having to repeat the analysis over and over.
For LC-MS, we require at least 10 µg or 50 µg of precipitated protein for nanoLC-MS or microLC-MS analysis, respectively.

Q: How pure does my protein have to be?
A: Again, as pure as you can get it. Remember, mass spectrometry is a physical technique, so it will detect ANY polypeptide material.

Q: Do I have to take special precautions when preparing my sample?
A: Try to work in clean conditions. The most frequent ubiquitous contaminant is keratin that covers virtually any object used by humans. Keratin is actually

one of the major components of common DUST. Wash well your gel plates. Wear powder free gloves when loading your gels and especially when cutting the band or spot. If possible, do the cutting under a laminar air flow (hood). Use clean micro-tubes.

Q: How should I prepare my sample from SDS-PAGE?
A: Run your gel, stain with Coomassie Blue, silver stain or a fluorescent dye (e.g. SYPRO ruby). You should make sure that you are using a MS-compatible stain (see **"What staining protocol can I use?"** below).
Gels should be distained as well as possible, since stain may interfere with data acquisition.
Excise you protein band(s) of interest and cut it into approximately 1-2mm^3 gel-cubes; avoid including diffuse stained edges to the band. Alternatively you can use a clean pipette tip to punch out a round gel piece (see **"How big can the gel slice be?"** below).
Place gel cubes into a washed, plain 1.5 mL micro-tube (a low-protein binding micro-tube is preferable, when available). Do not use any tubes with O-rings or gaskets. DO NOT use autoclaved tubes or "coated for PCR" tubes. Alternatively, if you are submitting many samples, you can use low-protein binding plates. No additional solution is needed to cover the gel band. Do not parafilm the tube. The gel band will remain moist and can be stored in a -20 degree freezer or shipped to us. Samples should be brought/shipped to us on dry ice or in the presence of cold-packs (if you have doubts, please contact us). Put your samples in a bag instead of loose in the containing box as vial tops can come loose.
If you are in the Oeiras campus or close by, please bring us your samples preferably in the morning.

Please ship your samples to:
UniMS – A/C Catarina Correia
Edifício ITQB
Av. da República
Estação Agronómica Nacional
2780-157 Oeiras
Portugal

Q: How should I prepare my sample from LC-MS runs?
A: We require at least 10 µg or 50 µg of precipitated protein for nanoLC-MS or microLC-MS analysis, respectively.
You should perform your protein extraction, using the protocol that better suits your proposes and determine protein concentration. Then perform a precipitation step on the required total protein amount, using low protein

binding 1.5 mL micro-tubes. Tubes should be brought/shipped to us on dry ice or in the presence of cold-packs (if you have doubts, please contact us). Put your samples in a bag instead of loose in the containing box as vial tops can come loose.
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Q: What staining protocol can I use?
A: All most common staining methods are compatible with subsequent analysis. Avoid any fixing step employing glutaraldehyde or paraformaldehyde (very commonly used in silver-staining procedures). In general the less you fix, the better (but this is often a trade-off with staining quality). Colloidal Coomassie Blue or rapid Silver staining usually give the best results. Or, if you can afford it, the fluorescent Sypro Ruby stain.

Q: How big can the gel slice be?
A: A good way to excise proteins is to use a clean pipette tip that has been cut so to give an aperture of 1.0-1.5 mm in diameter and punch out a round gel piece. Make sure the gel is well hydrated when you cut. Cutting the gel "under water" is a good idea, it also helps reducing keratin contamination. Cutting is a tricky procedure and you should practice on a test gel before going for a real sample.

Q: My protein is spread over a large surface. Should I cut it all?
A: Unfortunately protein concentration in the gel is a critical factor for the efficiency of the digestion. 0.1 µg of protein on a 1 mm^2 surface will give a better yield than 0.5 ug on 20 mm^2. In addition, processing large gel volumes poses a number of technical problems. Therefore, try to maximize protein concentration and only cut the "darkest" portion of your silver or Coomassie spot.