Max Perutz Laboratories - Mass Spectrometry Facility

General sample submission & preparation guidelines

A general note

We regard it as our job to help you tackling your experimental question in the best possible and most effective way. We will treat your samples with utmost care and apply the most sensible procedures existing to our knowledge to provide you with the information that you need. Despite these efforts we cannot guarantee that all analyses will turn out to be successful. Apart from technical problems (contamination by keratins, detergents, etc.) which we try to avoid, it is still possible that your peptide or protein of interest cannot be identified because it is below the detection limit or because it has properties which are not suitable for mass spectrometry. If such a situation occurs we consider it important to take the time to explain what the difficulties are and to see if we can find another solution together. This means whenever there is a problem, when there is something that is not clear to you, or when you have just a really interesting idea in mind that you would like to have feedback on, please contact us – we consider this an important part of our service and by no means a "disturbance".

1. Sample submission guidelines

1.1 Submission schedule and turn-around times

Please always contact us before submitting samples so that we can plan our workload accordingly. We will then let you know when the samples can be submitted and also give an estimate when they will be processed. **Standard samples** for simple workflows (like gel IDs) **can be submitted after short notice**, for all **other samples (e.g. CoIPs on beads, etc.) please inform us at least three days in advance**.

When submitting **samples which are important to be processed immediately** (for example samples on beads) please consider that sample processing before digestion can take several hours, followed by an overnight digest and subsequent steps the next day. Consequently, such samples **should not be submitted after 2 pm and not on Fridays or before holidays**.

We aim to return data for simple workflows within 7 to 10 workdays. However, turnaround times certainly depend on the length of the sample queue and the complexity of the analysis, we will try to provide a realistic estimate for this during submission. Standard workflow samples are processed in the order of their arrival. We will not prioritize samples from specific users.

1.2 Sample submission

To maximize the number of successful measurements, please consider the following points before submitting your sample:

- Please download and complete the **submission form** and send it to us on the day of submission or before. It is in your own interest to provide as much information as available about the nature of the samples and about your requirements. The more we know about your sample the easier it is to choose the best suited workflow and to avoid common problems.
- Please have a thorough look on our **sample preparation guidelines** below to avoid the most common problems or contaminations.
- In case of samples separated by gel electrophoresis, please provide a **scan of the stained gel with the submitted bands/spots labeled**. You can excise bands or spots yourself before submission or bring the gel to the facility and excise them with our help.
- In case of samples in solution, it is important for us to know **the exact composition of the solvent** and the amount or concentration of the protein. If possible please run a gel with a small aliquot of the sample and submit a photo or scan of it.
- **Label your sample tube** with a permanent marker. The label should contain the name of the sample owner and a short identification number or code that allows unambiguous identification for you and us.
- For the identification of proteins derived from constructs please **include the exact sequence, including tags, cleavage sites, etc.** which you expect to be present in the protein in the submission form or send it as a separate file.
- To make sure that we do not harm our LC-MS system and to estimate the injection amount we run a small fraction of each sample on a QC system beforehand. We reserve the right not to measure particular samples if the QC system indicates a contamination with problematic substances.
- **Radioactively labeled samples must not be measured on our systems and cannot be accepted.**
- **We only accept biologically inactivated material.**
- **^{15}N-labeled proteins** are not suitable for routine analysis. The increased complexity of the isotopic envelope and incomplete labeling, make peptide identification from these samples challenging. Please contact us if you would like to work with ^{15}N labeled proteins.

2. Sample preparation guidelines

2.1 General considerations

Inappropriate sample handling is the most common source for contamination and other problems in the downstream analysis. Please read the following considerations carefully and keep them in mind when handling your samples and gels – the quality of your data depends on it.

- Contaminations can occur in all phases of sample handling.
- Dust particles contain high levels of proteinaceous **contaminations** (hair and skin particles, wool fibers, etc.) and as a consequence you should try to work as cleanly as possible. Wash or clean all equipment very thoroughly before use, filter solutions, wear (powder free nitrile) gloves and lab coats at each step, use chemicals and solutions dedicated to "mass spectrometry", use new pipette tips instead of autoclaved ones, and do not touch the vials at any stage of the sample preparation without gloves.
- Low quality **plasticware** or solvents stored in plastic containers can severely contaminate your sample with plasticizers (polymers), impede the analysis or make it completely impossible. Contact us if you are in doubt of what to use.
- Bear in mind that "clean" **glassware** which comes directly from the dishwasher may contain considerable amounts of detergents.
- In case you are not sure about the "cleanliness" of your laboratory, it is possible to use our laminar flow for particular preparation steps (e.g. gel band cutting). Please contact us in advance so that we can arrange the hood for you or help you with your setup.

2.2 Method-specific points to consider

2.2.1 Proteins separated by gel electrophoresis

There are several methods to visualize proteins after gel electrophoresis. Unfortunately, not all of them are compatible with mass spectrometry. **Coomassie staining** techniques are in general ideal. However, there are some cases when higher sensitivity is needed. Please note that silver staining applying glutaraldehyde in the sensitizing solution is not compatible with mass spectrometry. For this reason we recommend using Coomassie staining or an alternative **silver staining protocol** (please contact us). In case of silver staining do not overstain the gel, keep the background clear. In our experience, overstaining reduces the yield of identifiable peptides from the sample substantially.

Carry out all staining steps in a **clean and closed container** and cut the bands/spots under a laminar flow hood. Do not use containers that have been used for Western blocking before (milk powder, etc.) and only use clean (filtered) buffers.

Bands or spots should be cut with a clean scalpel or razor blade and stored in a clean and labeled tube. Keep the volume of the empty gel matrix as small as possible, cut away unstained gel material. Samples with volumes exceeding the size of typical bands should be discussed with the facility team before submission.

Submit the samples as soon as possible; freeze them only if you can guarantee that they will not thaw during the transfer to our lab. If you send the sample via mail, please contact us before and send it on dry ice.

2.2.2 Protein solutions or pellets

Please note, that samples should be free of detergents (e.g. SDS, Triton, etc.), high concentrations of glycerol, polymers such as PEG, and radioactive labels.

Use **detergents** very carefully during sample preparation, they cannot be removed by dialysis and only some of them can be removed by other means but often with considerable loss of sample. In all cases please let us know which detergents you used and how, so that we can see if we have to do something about it. Injecting detergents into an LC-MS system does not only hamper the current analysis but may severely reduce overall performance requiring long and costly cleaning procedures.

Fresh protein solutions should be delivered on ice as soon as possible or snap frozen in liquid nitrogen and delivered on dry ice. If you send the sample via mail, please contact us before and send it on enough dry ice to prevent thawing (avoid sending over the weekend and public holidays and take into account customs regulations if you send it from abroad).

2.2.3 Proteins bound to beads

If you isolate your proteins with beads coated with proteins (e.g. antibodies, calmodulin, etc.), please contact us before you submit the sample. If the proteins are bound to beads, please submit the samples in a fresh state, do not freeze them! Please also read through the considerations for protein solutions above as they similarly apply to bead samples.