GUIDELINES AND PROTOCOLS

Protein Characterization

Protein/peptide analysis by HPLC/MS and MS/MS

Please make sure you have a minimum of 20 µL of protein/peptide sample, at a concentration of at least 5 ng/µL. However, for mass spectrometry analysis the detection limits are on the molar basis, so strictly dependent on the molecular weight of the protein/peptide of interest.

If the sample is in solution, please indicate the composition of the buffer and the concentration of the peptide/protein. Provide the sample in dry ice.

If the sample is a pellet, please indicate the amount of the peptide/protein and the presence of salts/detergents. The sample can arrive at room temperature.

Intact protein MW determination by nanospray off line MS

If the determination of the molecular weight has to be directly performed by mass spectrometry, without any preliminary purification/desalting step, it is important to avoid the presence of salts/detergents, which can interfere with the ionisation process during the mass analysis.

If the sample is in solution, please indicate the composition of the buffer and the concentration of the peptide/protein. Provide the sample in dry ice.

If the sample is a pellet, please indicate the amount of the peptide/protein and the presence of salts/detergents. The sample can arrive at room temperature.

The amount required for this activity is approximately 2 pmol.

Protein Identification

This guideline is meant to ensure an acceptable level of quality in the samples submitted for mass spectrometry analysis. They can come in:

1. gel slices
2. liquid solutions

Reduce contamination from keratins and other proteins: contamination primarily derives from fingers and hair of investigators in the form of proteins, lab dust should be avoided.

- Always wear gloves when handling samples
- Never touch gels directly with bare fingers or leave gels exposed to dust in the lab atmosphere. This includes any apparatus involved in the preparation of gels or any surfaces that come into contact with gels. For example, use ethanol-soaked kimwipes to clean working area/apparatus.
- Tubes, pipette tips and other supplies that come into contact with the sample should come from keratin-free, clean closed plastic bags/boxes prior to use.

Proteins in gel slices

It is strongly recommended that you use Coomassie Brilliant blue or Colloidal Coomassie blue stain (detection limits between 10 and 50 ng). In the range of higher sensitivity, we recommend to use mass spectrometry-compatible Silver staining protocol or Sypro-Ruby protocol (Bio-Rad or Invitrogen instructions) with detection limits between 1-5 ng. The latter is a fluorescent dye, mass compatible, as sensitive as Ag staining but much easier to use.

Protein stains detect total protein, while the mass spectrometer detects proteins individually. Often a gel band contains several proteins, rendering the band detectable with Ag stain, but most likely not detectable by the mass spectrometer. For this reason we suggest the use of Coomassie stain, particularly for PTM-definition by mass spectrometry. The entire gel slice, containing the bands to be analyzed, has to be stored in a bag with 1% acetic acid.

Proteins in liquid solutions

The buffer should contain no urea, no detergents. Funk et al. (2005) [Rapid Commun Mass Spectrom; 19, 2986-2988] reported the maximum tolerable detergent concentration of commonly used detergents for MALDI-TOF/MS and ESI-TOF/MS.

Sample collection for biomarker profiling, quantitative protein profiling and metabolomics/lipidomics

We strongly recommend contacting the ProMeFa staff before starting any study

1. Except for serum: Leaving any kind of sample at room temperature during processing (even if only for a few minutes) should be avoided. Leaving blood, serum or urine samples on ice bath (2-4°C) during processing should not exceed a total of 30 minutes.
2. If a previous core biopsy site is noted in the surgical specimen, cores should not be taken from near that site. Core biopsies alter the biology of tissue (e.g. genes involved in wound healing are very similar to those involved in cancer progression)
3. Prior to extracting biomolecules from fresh/frozen tissue, core samples should be histologically evaluated in order to reveal the presence of necrotic or inflammatory material.
4. Whether or not to perform laser-capture microdissection, or its equivalent, on fresh tissue for proteomics depends on the intended analysis. For examining global expression of proteins (i.e., in the stroma and epithelium), laser-capture microdissection should not be performed. If only information on the protein spectrum in the epithelium or stroma is sought, then laser-capture microdissection should be performed.

Details on storage

1. For proteomics, metabolomics and lipidomics, -80°C is preferred, although samples (except for tissues) can be stored at -20°C for up to 2 weeks without major problems. It is important to note that proteins are fragile macromolecules and are prone to degradation if not handled and stored correctly. Small molecules can be easily oxidized or degraded.
2. Samples should NEVER be thawed (especially when transferred between sites, or from -20°C to -80°C). Number of thawing needs to be recorded!
3. Samples should not be kept at -80°C AND THEN transferred and kept at -20°C.

Sampling for metabolomics analysis by Nuclear Magnetic Resonance (NMR)

Sampling and storage:

- Samples are generally collected in 1.5/2 mL eppendorf tubes, 15/50 mL falcon tubes or in cryovials; immediately after collection, the samples must be kept on ice (or approx 4°C) or snap frozen in liquid nitrogen and safety stored at 80°C for long time to avoid sample degradation.
- In case of murine and human samples, age, gender and fasting/diet state must be indicated.
- If replicates are being collected from patients, record the duration of sampling by labelling.
- Clearly label tubes with a unique labeling system, on both lids and sides of the tubes, with water resistant markers.
- Samples should never be thawed.