Sample Preparation Instructions

Mass Spectrometry & Proteomics Core Faciity

General features

1. Work in a clean environment.
2. Avoid keratin contamination by using gloves and lab coats.
3. Do not use autoclaved material.
4. Wash all the material with milliQ water and MeOH (high quality) and let them dry before use.
5. Use LC-MS grade reagents.
6. If possible use low-binding eppendorf.
7. Use simple and well-recognized tube labeling. We recommend using consecutive numbers.

Samples for in-gel digestion

1. When possible run a 0.75 mm thickness gel.
2. Use clean recipients for staining. Close them during staining period to avoid contamination.
3. NEVER use recipients that were previously used for Western blot.
4. Use a mass spectrometry compatible protocol for gel staining.
5. If you use fluorescent stain do not cut the bands manually by using a transilluminator.
6. Cut only the stained region. The higher protein concentration and the lower acrylamide presence, the better protein identification.
7. When possible cut the gel spots or bands in a laminar flow hood to avoid keratin contamination.
8. Store bands at 4ºC without water or other solvents.

Samples for in-liquid digestion

1. There are some reagents not compatible with mass spectrometry. Below are listed the most commonly used and not compatible with LC-MS. Please tell us the reagents you use for sample preparation.
    - Igpal/NP40 is almost impossible to remove and shows large polymers along all the chromatogram, so we will suggest changing it for N-octyl-β-glucopyranoside or avoiding any detergent if possible.
    - The RNAse is a protein that could mask the proteins of interest.
    - When possible avoid glycerol.

2. For IP experiments a negative and a positive control are needed to find out nonspecific interactions. We also recommend running a SDS-PAGE gel with 10% of the sample to have an idea how the IP works.
3. The amount of sample required for protein identification depends on sample complexity and purity. For complex samples at least 1nM is required.

Intact proteins

1. An efficient ionization (ESI or nanoESI) of proteins depends on their length, and physicochemical properties (pKa, solubility, structure and hydrophobicity). It is possible to work under denaturing or under non-denaturing conditions
    - Denaturing conditions: proteins are solubilized generally in a 1:1 mixture of acetonitrile/water or methanol/water and 1% formic acid is added to enhance ionization (all solvents must be MS quality). Depending on their purity, proteins are analyzed by infusion or by LC-MS. 1μM to 10 μM protein concentration is required (volume: 10-100 μl). Low dynamic ranges are normally achieved and pre-purification is therefore required. Non-volatile salt removal is necessary (Zip-tips can be used). Maximum non-volatile salt accepted in the case of LC-MS experiments is 150 mM. Infusion experiments require complete salt removal.
    - Non-denaturing conditions: proteins or non-covalent protein complexes are solubilized in NH4OAC (10mM-1M). Salt removal is imperative and is done normally by dialysis or with Biospin columns. 5μM to 50 μM protein concentration is required.
2. Top-down (MS/MS experiments) can be performed on intact proteins to localize modifications or confirm amino acidic sequence. Protein length is limited to 30 kDa to perform MS/MS in the gas phase with the aim of entirely sequencing the protein. Middle-down strategies are otherwise used. Denaturing conditions are used in this case. 5μM to 10 μM protein concentration is required (100 μl).
3. Top-down can also be used for protein identification (up to 100-150 kDa). In this case protein coverage is low (this strategy is very much in underdevelopment with respect to the classical bottom-up approach (proteomics) although useful for protein isoform identification)

If you have any question please contact marta.vilaseca@irbbarcelona.org