SERVICIO DE PROTEÓMICA Y GENÓMICA

INSTRUCTIONS FOR SENDING SAMPLES TO THE SERVICE

It is necessary to fill in the application form available on the website of the Service that can be delivered to the laboratory by hand or attach it in the sending of the samples.

https://www.cib.csic.es/facilities/scientific-facilities/proteomics-and-genomics

1) Molecular mass determination by MALDI-TOF.

• Solutions should be free of salts, detergents and contaminants that may interfere with mass spectrometry. The presence, among others, of compounds such as NaCl, KCl, CaCl2, DMSO, DMF, Glicerol, Polyethylene glycol, SDS, Triton X-100, Tween 80, N-octylglycoside, Urea and Guanidinium Chloride should be avoided. Do not interfere with mass spectrometry composites such as β-Mercaptoethanol, DTT, Trifluoroacetic Acid, Formic Acid, Acetic Acid, HCl, or volatile organic solvents. The reagents to use should be purity grade for HPLC and the water should be milli-Q.

To avoid these issues we recommend:

- Dialysis of the sample.
- Desalting the sample using Zip-Tip, OMIX, etc. (tips with C4 or C18 reverse-phase columns).
- Providing the sample as concentrated as possible to perform dilution with the appropiated solvents.

• For mass spectrometry analysis, the concentration of peptides must be in the order of magnitude of femtomoles. Some picomoles are needed for proteins of about 20 kDa, and tens of picomoles are required for higher molecular mass.

The mass range for proteins is up to about 40 kDa and the mass error is approximately the average mass of one amino acid. The mass error for peptides is about 80 ppm.

2) Protein identification by peptide mass fingerprinting (PMF).

• Samples may consist of single-dimensional gel isolated protein bands (SDS-PAGE) or protein stains from two-dimensional gels (IEF+SDSPAGE).

The concentration required to analyze a protein by PMF is that in which it is possible to visualize the isolated protein in a polyacrylamide gel by any of the above methods (5 fmol/µl to 1 pmol/µl).

• Bands from monodimensional gels may contain a mixture of proteins that will increase the difficulty of the identification process by MALDI-TOF/TOF mass spectrometry. In these cases samples can be analyzed using liquid chromatography coupled to mass spectrometry, nLC-MS/MS, (LTQ Orbitrap Velos or QExactive).

• Throughout the process of obtaining the sample for subsequent analysis by mass spectrometry, extreme cleanliness of the material used must be taken (glass, plastic, etc., washed with milli-Q water, not autoclaved).

• It is ESSENTIAL to wear gloves (without talcum powder, dust) all the time to avoid contamination with keratins, which are found in hair, skin, certain types of clothing (wool), etc. ALWAYS prevent contact with bare hands, loose hair and skin. Keratins are very ubiquitous and interfere with digestion and identification of proteins of interest. Low identification levels may be due to keratin contamination.

3) 1D ELECTROFORESIS.

• It is necessary to use freshly prepared solutions, using milli-Q or HPLC-grade H2O for all electrophoresis and staining solutions.

• Gels should be polymerized overnight to avoid reactivity of unpolymerized acrylamide with proteins during electrophoresis.

• CLEAN containers must be used to stain the gels, without BSA or casein from previous western blots, etc. Otherwise, these proteins will make it impossible to correctly identify the proteins of interest.

• Be sure to perform a staining protocol compatible with mass spectrometry. Many protocols can cause proteins to be unsuitable for mass spectrometry analysis. Gels can be revealed with common Coomassie Blue or Colloidal Coomassie stains. It is necessary to include molecular mass standards in one lane of the gel.

Stained gels should be stored in 1% acetic acid or water and sent to the proteomics service as soon as possible. It is preferable to carry out the acquisition of the images of the gel and the extraction of the bands of interest in the service.

If you decide otherwise, you have to be extremely careful and make sure to use absolutely clean surfaces and new blades or scalpels. Even traces of contaminants could cancel any attempt to analyze the proteins. If the samples are bands of one-dimensional gel, they should be trimmed (approx. 1.5-2.0 mm) with a clean scalpel and forceps; if they are protein spots from a two-dimensional gel, they can be cut out with a micropipette tip, avoiding unnecessary amounts of gel and proteins mixture. The gel portion containing the protein spot is placed in an eppendorf brand tube and covered with milli-Q water. A file with the image of the gel has to be sent.

• Samples from external users. Samples must be sent lyophilized or frozen in solution (volume should not exceed 20 µl). In the latter case they must contain protease inhibitors.

Alternatively, samples can be shipped in electrophoresis loading buffer (volume should not exceed 30 µl).

4) Protein identification by LC-MS/MS.

User instructions:

- The sample can be in gel or in solution.
- The minimum amount of sample depends on the objectives of the work (consult with the staff of the Proteomics Service).
- Protein extracts should be stored at -70 °C and never re-freeze after thawing.
- The amount of total protein should be estimated by some colorimetric method and/or running an SDS-PAGE gel. The recommendations in point 3 (ELECTROFORESIS 1D) shall be followed in the preparation of the gels.

For the analysis of Immunoprecipitations (IP), Pull-Down, subproteomes or proteomes, contact the technical staff of the Service to design a mass spectrometry analysis protocol according to the objectives of the work and/or the characteristics of the sample (origin, amount of protein, solvents, etc.).

Note:

The service is not responsible for the results obtained if the samples do not meet the conditions specified in these instructions.